Dissertation on

HEMOSTATIC STATUS IN HIV INFECTED INDIVIDUALS

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CERTIFICATE

This is to certify that the dissertation entitled **"Hemostatic status in HIV infected individuals"**is a bonafide original work of **Dr. YOUMASH.V.P,** in partial fulfillment of the requirements for M.D. Branch– I (General Medicine) Examination of the Tamil Nadu Dr. M.G.R Medical University to be held in APRIL 2012 under my guidance and supervision during the period of April 2011-November 2011.

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DECLARATION

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ABBREVIATIONS

AIDS - Acquired immuno deficiency syndrome

ART - Anti Retroviral Therapy

aPTT - Activated partial thromboplastin time

AZT - Zidovudine

APL - Antiphospholipid

cART - Combination Anti-retroviral therapy

CD4 - Cluster of differentiation

dRVVT - dilute Russell viper venom test

DVT - Deep venous thrombosis

DN-MK - Denuded Megakaryocyte Nuclei

ELISA - Enzyme Linked Immunosorbent Assay

HAART - Highly active antiretroviral therapy

HbsAg - Hepatitis B surface antigen

HIV - Human Immunodeficiency Virus

HTLV - Human T cell lymphotropic virus

HbsAg - Hepatitis B surface antigen

HBV - Hepatitis B virus

HCV - Hepatitis C virus

Ig - Immunoglobulin

ITP - Idiopathic thrombocytopenic purpura

INR - International Normalized Ratio

ISI - International Sensitivity Index

IRP - International Reference Preparation

LAV - Lymphadenopathy associated virus

LDH - Lactate dehydrogenase

LAC - Lupus anticoagulant

NACO - National AIDS Control Organisation

NACP - National AIDS Control Programme

NNRTI - Non nucleoside Reverse Transcriptase Inhibitor

PLT - Platelet

PLHA - People Living with HIV-AIDS

PMTCT - Prevention of mother-to-child transmission

PT - Prothrombin time

PTT - Partial thromboplastin time

RHD - Rheumatic heart disease

SACS - State AIDS Control Societies

SE - Standard error

SEM - Standard error of mean

TB - Tuberculosis

TTP - Thrombotic thrombocytopenic purpura

UNDP - United Nations Development Programme

VTE - Venous thromboembolism

WHO - World Health Organisation

INTRODUCTION

With 39.5 million people worldwide living with human immunodeficiency virus (HIV) infection, acquired immune deficiency syndrome(AIDS) is one of the most destructive pandemics in recorded history. AIDS is the late stage of an infection that is generally acknowledged to be caused by the HIV. HIV is a retrovirus that attacks and destroys the lymphocytes. The targeted destruction weakens the body's immune system and makes the infected person susceptible to infections and diseases that ordinarily would not be life threatening. AIDS is considered a blood-borne, sexually transmitted disease because HIV spreads through contact with blood, semen, or vaginal fluids from an infected person.[1]

Discovery of HIV[1]

In September 1983, Luc Montagnier and researchers at the Pasteur Institute in Paris, France, isolated and identified a retrovirus and named it as Lymphadenopathy associated virus (LAV). Eight months later Gallo's group isolated the same virus in AIDS patients, which they called HTLV-III. LAV and HTLV-III were found to be identical and are now referred to as HIV.

HIV/AIDS IN INDIA

Prevalence of HIV in India is 0.29% (NACO). 61% are males and 39% females. Most common mode of transmission in India is heterosexual which comprises 87%. It is increasingly diagnosed in females without apparent risk

factors. According to a study in the British Medical Journal, India has a population of approximately 1.4-1.6 million people living with HIV/AIDS[2]. The estimated number of HIV infections in India has declined drastically in recent years—from 5.5 million in 2005 to below 2.5 million in 2007. These new figures are supported by the WHO and UNAIDS[3]. According to the United Nations 2011 AIDS report, there has been a 50% decline in the number of new HIV infections in the last 10 years in India.[4]

HISTORY

The first known case of HIV was diagnosed by Dr. Suniti Solmon in 1986 amongst female sex workers in Madras[5]. Later that year, sex workers began showing signs of this deadly disease. At that time, foreigners in India were travelling in and out of the country. It is thought that these foreigners were the ones responsible for the first infections. About 135 more cases came into light by 1987. Among these 14 had already progressed to AIDS. Prevalence in high risk groups reached above 5% by 1990.

Setting up HIV screening centres was the first step taken by the government to screen its citizens and the blood bank. To control the spread of the virus, the Indian government set up the National AIDS Control Programme (NACP) in 1987 to co-ordinate national responses such as blood screening and health education. In 1992, the government set up the National AIDS Control

Organisation (NACO) to oversee policies and prevention and control programmes relating to HIV and AIDS. The State AIDS Control Societies (SACS) was set up in 25 societies and 7 union territories to improve blood safety. In 1999, the second phase of the National AIDS Control Programme (NACP II) was introduced to decrease the reach of HIV by promoting behaviour change. The prevention of mother-to-child transmission programme (PMTCT) and the provision of antiretroviral treatment were materialized. In 2007, the third phase of the National AIDS Control Programme (NACP III) targeted the high-risk groups, conducted outreach programmes, amongst others.

EPIDEMIOLOGY

Despite being home to the world's second-largest population suffering from HIV, the AIDS prevalence rate in India is lower than in many other countries. In 2007, India's AIDS prevalence rate stood at approximately 0.30%—the 89th highest in the world.[7] As per UNDP's report in 2010, India had 2.39 million HIV population at the end of 2009, up from 2.27 million in 2008. Adult prevalence also rose from 0.29% in 2008 to 0.31% in 2009[6].The states with high HIV prevalence rates include Manipur (1.40%), Andhra Pradesh (0.90%), Mizoram (0.81%), Nagaland (0.78%), Karnataka (0.63%) and Maharashtra (0.55%).In the most severely affected state, Maharashtra, seropositivity was 60% among sex workers, 14–60% in sentinel STD clinics, and over 2% among women attending antenatal clinics (NACO, 1999, p. 4).[8]

HEMOSTATIC ABNORMALITIES[9]

HIV infection is becoming more complex. With the introduction of cART, HIV infection is slowly evolving into a chronic disease. Highly active antiretroviral therapy (HAART) has greatly reduced the risk of early death from opportunistic infections and extended the lifespan of people infected with the HIV. Thus, many complications and organic damage in the HIV-infected population emerge. In the recent years, hemostatic abnormalities have gained their significance in HIV infection. HIV-related thrombocytopenia is the most common hemostatic disorder with a high morbidity and affects patients from every risk group independently of age, sex, or stage of infection. Two mechanisms are responsible for this are bone marrow failure and immunological disorders, namely, circulating immune complex deposited on the platelet membrane and the production of auto-antibodies directed against platelets. The treatment of choice is cART. In addition, there are some abnormalities in the fluid phase of the coagulation cascade which can produce bleeding or thrombosis in the HIV patient. The most common are a prolonged partially activated thromboplastin time test, the production of a lupus anticoagulant and anticardiolipin antibodies, and several abnormalities in the natural-occurring anticoagulants. The knowledge of these hemostatic abnormalities and its early identification in the HIV seropositive patient allows a more rational care of these patients.[9]

AIMS AND OBJECTIVES

1. To analyse the hemostatic status of HIV infected individuals irrespective of their ART status.

2. To analyse the association of CD4 count, WHO staging, cART intake, and comorbidities independently with the hemostatic parameters.

REVIEW OF LITERATURE

Disorders of the hematopoietic system including lymphadenopathy, anemia, leukopenia, and/or thrombocytopenia are common throughout the course of HIV infection and may be the direct result of HIV, manifestations of secondary infections and neoplasms, or side effects of therapy. Initiation of cART will lead to reversal of most hematologic complications that are the direct result of HIV infection.[10]

Causes of bone marrow suppression in patients with HIV infection[10]	
• HIV infection • Mycobacterial infections • Fungal infections • B19 parvovirus infection • Lymphoma	• <u>Medications</u> Zidovudine Dapsone Trimethoprim/sulfamethoxazole Pyrimethamine 5-Flucytosine Ganciclovir Interferon Trimetrexate Foscarnet

HEMOSTATIC ABNORMALITIES IN HIV

THROMBOCYTOPENIA

Thrombocytopenia is relatively common during the course of HIV infection, occuring in approximately 40% of patients and serving as the first symptom or sign of infection in approximately 10%[11,12]. Severe thrombocytopenia was first observed in contaminated homosexual males. Thrombocytopenia may be an early consequence of HIV infection. Approximately 3% of patients with untreated HIV infection and CD4+ T cell counts >400 cells/microlitre have platelet counts <1,50,000/mm^3. For untreated patients with CD4+ T cell counts <400 cells/microlitre, this incidence increases to 10%. In patients receiving ART, thrombocytopenia is associated with hepatitis C, cirrhosis, and ongoing high-level HIV replication. After controlling for multiple factors, thrombocytopenia was significantly associated with shorter survival (risk ratio 1.7)[12].

HIV RELATED IMMUNE THROMBOCYTOPENIC PURPURA

Clinically, thrombocytopenia in patients with HIV resembles the thrombocytopenia seen in patients with idiopathic thrombocytopenic purpura. In patients with early stage HIV infection with intact CD4 counts, low viral load, no hepatosplenomegaly or adenopathy, and an otherwise normal complete blood count, HIV-associated immune thrombocytopenic purpura is the most likely

diagnosis. With progression of the underlying HIV disease, secondary causes of thrombocytopenia should receive greater consideration[12].

The two major components of the pathophysiology of HIV-associated thrombocytopenia are immune-mediated destruction of platelets, and a defect in bone marrow production as a result of the interaction between HIV and megakaryocytes through several pathways, which causes suppression of platelet production. Often both components can be present simultaneously. Elevated levels of platelet-associated Ig G and/or circulating immune complexes often indicate that immune-mediated destruction of platelets in HIV-infected individuals with thrombocytopenia is occurring. These patients may have a marked increase of platelet-associated Ig G, Ig M, C3, and C4 levels. The immune complexes are thought to contain anti-HIV gp120 and anti-antibodies (anti-idiotypes) directed against the anti-HIV antibodies. In a few patients, cross-reactivity of antibodies against HIV gp120/gp160 with platelet glycoprotein GPIIb/IIIa has been shown.[11] Because megakaryocytes are infected by HIV, the "anti-platelet" antibodies could also be directed against HIV proteins expressed on megakaryocytes or platelets. Although many patients have immune-mediated platelet destruction as an important or predominant cause of their thrombocytopenia, it is not thought to be helpful diagnostically to obtain "platelet antibodies" in the evaluation of a thrombocytopenic HIV-infected patient because the antibody tests have both high false-positive and false-negative rates.[12]

Human megakaryocytes bear a CD4+ receptor capable of binding HIV[13] and HIV-1 can be internalized by human megakacyocytes[14].The HIV-1 coreceptor CXCR4 is present on megakayocytic progenitors, megakaryocytes and platelets. Specific ultra structural damage in HIV infected megakaryocytes consisting of blebbing and vacuolization of surface membrane[15]. A casual observation that bone marrow biopsies of HIV-infected individuals seem to exhibit an unusually large number of denuded megakaryocyte nuclei (DN-MK) prompted a study comparing megakaryocyte nuclei of 20 HIV-seropositive individuals with those of 10 patients with HIV-negative idiopathic thrombocytopenic purpura and 10 hematologically normal subjects. In normal marrows the number of DN-MK average 2.1 $\pm$ 0.5 SE per 10 low power field. In patients with ITP the average number was 6.5 $\pm$ 1.4 SEM, whereas HIV-ITP marrows had an average of 42.5 $\pm$ 3.7 SEM. Electron microscopy of AIDS megakaryocytes exhibited ballooning of the peripheral zone to an extent not seen by us in any other myelodysplastic syndromes. These observations support the concept that the pathophysiology affecting platelets/megakaryocytes in HIV-infection should not be equated with the destructive process underlying other immune thrombocytopenias.[15]

In individuals with advanced HIV disease, other causes of thrombocytopenia, such as opportunistic infections, medication side effects, and infiltrative malignancies, are more likely. This is particularly true when there are associated physical examination findings or other cell line deficits.

Opportunistic infections can cause thrombocytopenia, especially histoplasmosis and cryptococcosis. Medications used in the management of HIV that are most commonly associated with thrombocytopenia include ganciclovir (48%), pentamidine (18%), and trimethoprim-sulfamethoxazole (3%). Rifabutin, clarithromycin, zidovudine, and didanosine are less commonly associated with thrombocytopenia.[12]

Experimental tests with potential future use in the diagnosis of HIV-associated thrombocytopenia include the following:

- Measurement of thrombopoietin levels: Thrombopoietin levels are almost always normal in HIV-associated thrombocytopenic purpura unless the disease has progressed to a very late stage and the number of marrow megakaryocytes is decreased.
- Measurement of glycocalicin: Glycocalicin is the carbohydrate-rich portion of platelet membrane glycoprotein Ib-*a* Symbol, which has been reported to be increased when there is increased platelet turnover.
- Platelet reticulocyte counts: Platelet reticulocyte counts are thought to reflect the percentage of young platelets.[12]

When these tests are available to the general practitioner, they may allow better estimation of the rate of platelet production.

TREATMENT FOR HIV RELATED THROMBOCYTOPENIA

Whatever the cause, it is very clear that the most effective medical approach to this problem has been the use of cART[10]. In a retrospective study, 15 patients with HIV-associated severe thrombocytopenia (platelet count < 50×10^9/l), mostly antiretroviral experienced (13/15), underwent HAART for at least 6 months (median 21; range 6–41 months) during which the platelet (PLT) count and plasmatic HIV-RNA were monitored. The PLT response was compared to that observed in previously treated patients with zidovudine (AZT) monotherapy. HAART induced a significant increase in the PLT count (P =0.01) within the third month which was sustained up to the sixth month of therapy. No severe thrombocytopenia relapse was observed among eight PLT responders followed for longer than 6 months (median 27; range 7–41 months). The PLT increase after HAART was similar to that observed with AZT monotherapy, but a greater number of HAART patients were antiretroviral-experienced. HAART determined a PLT response in 10/13 subjects whose thrombocytopenia had not improved after previous AZT monotherapy. After 6 months of HAART, a complete platelet response occurred more frequently in patients with undetectable plasma HIV-RNA levels (P=0.01).[19]

For patients with platelet counts <20,000/mm^3 ,a more aggressive approach combining IV Ig or anti-Rh Ig for an immediate response with cART for a more lasting response is appropriate. Rituximab has been used with some

success in otherwise refractory cases. Splenectomy is a rarely needed option and is reserved for patients refractory to medical management.

HIV RELATED THROMBOTIC THROMBOCYTOPENIC PURPURA

In patients with early HIV infection, thrombocytopenia has also been reported as a consequence of classic thrombotic thrombocytopenic purpura. This clinical syndrome, consisting of fever, thrombocytopenia, hemolytic anemia, and neurologic and renal dysfunction, is a rare complication of early HIV infection. HIV related TTP generally has milder cause and better response to therapy than classical TTP[16]. HIV can infect endothelial cells, and viral P24 antigen has been detected in splenic endothelial cells, spinal cord specimens and in bone marrow microvascular endothelial cells[17]. TNF-α and IL-1β are increased in HIV infection could potentially lead to increases in endothelial expression of adhesion molecules such as vascular cell adhesion molecule-1, intercellular adhesion molecule and E-selectin promoting localization of inflammatory cells to endothelium. Endothelial cells from small blood vessels undergo apoptosis when exposed to plasma from patients with TTP[18].As in other settings, the appropriate management is the use of salicylates and plasma exchange.[10]

THROMBOEMBOLISM

The incidence of venous thromboembolic disease such as deep-vein thrombosis or pulmonary embolus is approximately 1% per year in patients with

HIV infection. This is approximately 10 times higher than that seen in an age-matched population. Among the factors associated with clinical thrombosis are age over 45, history of an opportunistic infection, lower CD4 count and estrogen use. Abnormalities of the coagulation cascade including decreased protein S activity, increase in factor VIII, anticardiolipin antibodies, or lupus-like anticoagulant have been reported in more than 50% of patients with HIV infection.[10] Even if lupus anticoagulant could be evidenced in asymptomatic PLHAs, it frequently occurred during acute opportunistic infections such as Pneumocystis carinii.

In one study in which the protein S level was measured in 34 HIV-infected children with no previous history of thrombosis, decreased levels of protein S (total and functional) were found in 76.5% of the patients.[21]These decreased levels of protein S were more prevalent in subjects with CD4 counts <200 cells/mm^3. Long-term studies are needed to determine the cumulative risk of thrombotic complications caused by these abnormalities and to better describe the complete coagulation profile in these patients.[12]

Abnormalities of the fibrinolytic system were also reported, such as increased levels of both tissue-type plasminogen activator and type 1 plasminogen activator inhibitor or decreased levels of histidine-rich glycoprotein. Even if the acute phase response could play a key-role, the pathogenesis of these abnormalities is not fully understood, so far. In addition, their clinical consequences have not been extensively investigated, but

hemorrhage appeared to be uncommon. Moreover, D-Dimer levels were found increased in HIV-infected patients, suggesting that HIV-infection might be associated with a prothrombotic state, which could lead to clinical thrombosis in some HIV-infected patients (2%).[20] Recent epidemiological studies emphasize the increased incidence of thromboembolic events including myocardial infarction in the HIV-infected population after the introduction of highly active antiretroviral therapy. The use of protease inhibitors in particular is implicated.[23]

CHRONICITY ASSOCIATED WITH VENOUS THROMBOTIC DISEASE:

HIV infection has changed into a chronic infection with the chance of developing long-term complications. Vascular complications are frequently reported in the current literature. HIV and treatment by highly active antiretroviral therapy (HAART) are associated with many cardiovascular risk factors. An increased risk of arterial cardiovascular complications was found in a number of studies. However, data about the risk of venous thrombotic disease (VTE), including potentially fatal conditions like pulmonary embolism, were limited. In a systematic review of the literature, ten relevant epidemiological studies were identified that investigated the risk of venous thrombotic disease in HIV-infected patients. The incidence was increased two- to tenfold in comparison with a healthy population of the same age. However, these studies were mainly retrospective cohort studies that were prone to selection bias,

confounding factors were not always mentioned and in all but three control populations were missing. An increased risk of venous thrombotic disease in HIV-infected patients could be explained by the presence of a hypercoagulable state, characterised by an increase in procoagulant factors, such as endothelial tissue factor expression and thrombogenic properties of microparticles, and a decrease in anticoagulant factors, including anti-thrombin III, Heparin cofactor II and the protein C pathway. Furthermore, the risk of VTE was associated with an increased risk of infections and autoimmune haemolytic anaemia, and was weakly associated with HAART.[22]

The coagulation abnormalities seem to correlate with the degree of immunosuppression, as indicated by a change in the CD4+ cell count. Abnormalities of protein C, including decreased levels of functional protein C or antigenic protein C, have been identified in patients infected with HIV.[24] Cases of antithrombin III deficiency in patients with HIV who experienced thrombotic events have been reported,[25] and most cases of antithrombin III deficiency are attributed to urinary loss (HIV-related nephropathy).[26] The prevalence of lupus anticoagulants and anticardiolipin antibodies in patients infected with HIV has been reported to be as high as 53% to 70% and 46% to 90%, respectively.[27] Although the association of lupus anticoagulants with thrombosis in HIV infection is rare, thrombotic complications, such as transient ischemic attack, mild thrombotic stroke, avascular necrosis, edema of skin, and

skin necrosis, have been documented in patients with HIV infection who have positive antiphospholipid antibodies.[28]

ANTI PHOSPHOLIPID ANTIBODIES:

In HIV infected persons, the presence of APL is associated neither with the stage of the disease, the CD4 cell count, the viral load nor with a prothrombotic state *in vivo*. Abuaf and colleagues [29] found that the presence of APL in HIV-infected patients was not associated with lupus anticoagulant, a biological false positive test for syphilis, thrombocytopenia or thrombosis. Moreover, APL found in HIV infection are not anti-β2-glycoprotein I antibodies[29-31.] On the contrary, APL encountered in systemic lupus erythematosus are associated with an increased thrombin generation[32] and with anti-β2-glycoprotein I antibodies [33]. Taken together, these demonstrate that APL encountered in HIV infection do not share the features of immune APL, and that the presence of APL does not warrant anticoagulant treatment in HIV-positive patients.

PROTEASE INHIBITORS AND VENOUS THROMBOSIS:

In a study for 42-month period, 28 adult male homosexuals with AIDS experienced 34 thrombotic events. All but three received HAART regimen, two a successful round of double nucleoside analogue therapy, and one patient received no treatment. Median age of group was 38.5 years (range, 24 to 56

years). Median time from HIV infection to thrombosis was 40.5 months (range, 3 to 108 months). No patient had previous thrombosis, family history of thrombosis, or prothrombotic conditions. There were 31 deep vein thromboses, two pulmonary thromboembolisms, and one renal vein thrombosis. Six patients had two thrombotic events. The rate of thrombosis during the 42-month study period was 1.52% (cumulative incidence = 0.30%/year), while the rate of thrombosis in 600 patients before the era of protease inhibitor therapy was 0.33% (cumulative incidence approximately 0.055%/year) ($p < 0.001$). Due to high incidence of thrombotic recurrences and hemorrhagic complications while using oral anticoagulants, acetylsalicylic acid was initiated; no thrombotic episodes were recorded while using this drug. Protein C and protein S deficiency were found in nine and two patients, respectively. Two patients had lupus anticoagulant and two activated protein C resistance (APCR) without Factor V Leiden mutation (APCR test was negative after initial screening). Fifteen patients had no thrombophilic abnormalities. These data suggest that protease inhibitors could be a risk factor for venous thrombosis not due to thrombophilic abnormalities but likely related to abnormalities in platelets or endothelium[23].

MATERIALS & METHODS

SUBJECTS

Patients with HIV infection attending ART clinic and those who are inpatients in Rajiv Gandhi Government General Hospital, Chennai.

PERIOD OF STUDY

6 months

DESIGN OF STUDY

Cross-sectional study

CONSENT

Informed consent from all the patients

ELIGIBILITY CRITERIA

1. All patients with HIV infection

2. HIV infection proven by ELISA & western blot assay.

EXCLUSION CRITERIA

- Patients with inherited bleeding disorders or on anticoagulants.
- Patients who had previous thrombosis, family history of thrombosis and prothrombotic conditions.
- Patients were excluded if they refused to become part of study
- Patients less than 14 years of age and pregnant women

METHODOLOGY

All patients with HIV infection attending Govt. General Hospital during the study period were evaluated for the hemostatic parameters in the study. The parameters were platelet count, prothrombin time, activated partial thromboplastin time, plasma fibrinogen and serum lactate dehydrogenase. Special investigations like D-Dimer and LAC were done when required. Absolute CD4 count done by flow cytometric analysis was obtained. They were staged as per the WHO clinical staging given by the National AIDS control organisation (NACO).

Investigation details

Complete history and thorough physical examination of HIV infected individuals and routine blood investigations and coagulation profile .

Data collection and methods

Collection of data as per proforma with consent from HIV infected patients in Medicine wards and OP department.

Analysis

Data analysed using statistical package-SPSS software

Conflict of interest : Nil.

Financial data : Nil

Normal Values of parameters Assessed [10]

	Adult Male	Adult Female
Haemoglobin	13.3 to 16.2 g/dl	12.0 to 15.8 g/dl
CD4 count	600-1500 cells/mm^3 [34]	
Platelet count	1.65 to 4.15 lakh / mm3	
Prothrombin time	12.7 to 15.4 seconds	
Activated partial thromboplastin time	26.3 to 39.4 seconds	
Serum lactate dehydrogenase	115 to 221 units/litre	
Plasma fibrinogen	233 to 496 mg/dl	
D-Dimer	0.22 to 0.74 microgram/ml	

LABORATORY EVALUATION OF HEMOSTASIS [40]

PLATELET COUNT

Modern hematology analyzers perform a platelet count by electrical impedance or light scattering techniques that are accurate to ±5% in the range of 1000 - 3,000,000 platelets/µl. A measurement of platelet volume is provided at the same time, as well as a platelet size distribution curve. Automated platelet counts can be affected by platelet aggregates due to spontaneous aggregation, cold agglutinins, EDTA anticoagulants ("spurious thrombocytopenia, pseudothrombocytopenia") or particulate debris, such as red or white cell fragments("spurious thrombocytosis").[35-37]In addition, hematology analyzers may overestimate the platelet count in severe thrombocytopenia.[(38)] Therefore, confirmation of atypical platelet counts by manual inspection of a peripheral smear is essential. If necessary, platelet counts can be performed in a hemocytometer by phase contrast microscopy to an accuracy of ±10-20%.

BLEEDING TIME

The Ivy skin bleeding time is an imprecise manual screening assay of primary hemostasis that was widely utilized in the past as a diagnostic assay for patients with suspected bruising and bleeding disorders, as a therapeutic guide in actively bleeding patients, and as a predictor of hemorrhage in the general population of patients undergoing surgery or invasive procedures[39].A blood

pressure cuff is placed on the upper arm and inflated to 40 mm Hg to provide uniform capillary pressure, and a standardized incision is made on the volar surface of the forearm with a standard cutting device, such as the Surgicut and the Triplett and Tip Tripper Bleeding Time Devices. Blood is wicked from the incision with filter paper at 30-second intervals until bleeding ceases. The result is reported in seconds as the bleeding time.[41,42]

The bleeding time is determined by many physiologic factors, including skin resistance, vascular tone and integrity, and platelet adhesion and aggregation. Thus, a prolonged bleeding time may reflect an intrinsic platelet function defect, Von Willebrand disease, vascular anomaly, or medications that affects platelet function, such as aspirin. If the actual bleeding time exceeds the expected bleeding time by five minutes, a platelet function defect may be suspected. Unfortunately, the precision, accuracy, and reproducibility of the bleeding time are severely impaired by factors such as the thickness and vascularity of the skin, the location of the incision, skin temperature, wound depth, and patient anxiety. Because of its imprecision, the bleeding time must be used with extreme caution in a patient care setting. The US Food & Drug Administration no longer accepts bleeding time data in patients as a surrogate marker for the evaluation of new hemostatic drugs, and it is no longer indicated for the preoperative screening for hemostatic defects[43-46].The routine utilization of the bleeding time for the diagnostic evaluation of patients with von

Willebrand disease, storage pool disorder, and other hereditary mucocutaneous hemorrhagic diseases has been questioned.[47]

PROTHROMBIN TIME

The PT provides a functional determination of the integrity of the extrinsic (tissue factor) pathway of coagulation and is sensitive to the vitamin-K dependent clotting factors (factors II, VII, IX, and X) as well as to factors of the common pathway (fibrinogen,prothrombin, factor V, factor X). The PT is a widely used laboratory assay for the detection of inherited or acquired coagulation defects related to the extrinsic pathway of coagulation, and is the standard test for monitoring oral anticoagulation therapy(coumadin).[48,49]In the PT an aliquot of test platelet-poor plasma is incubated at $37^{o}C$ with a reagent containing a tissue factor, phospholipid (thromboplastin), and $CaCl_2$. The time required for clot formation is then measured by one of a variety of techniques (photo-optical, electromechanical,etc.). The result is reported in seconds (prothrombin time), or as a ratio compared to the laboratory mean normal control (prothrombin ratio, PTR). The PT is critically dependent on the characteristics of the thromboplastin used in the assay, as well as manner of blood coagulation, the type of container, the type of anticoagulant, specimen transport and storage conditions, incubation time and temperature, assay reagents, and the method of end point detection. This means that patients on

coumadin will have different clotting times when tested in different laboratories, so a means of standardization of results must be employed.

The International Normalized Ratio (INR) was introduced by the World Health Organization (WHO) in the early 1980's as a means of standardizing PT results.[50] For this purpose, a very responsive batch of human brain extract was designated as the first International Reference Preparation (IRP), and a correction factor (International Sensitivity Index, ISI) was developed to correlate the sensitivity of commercial thromboplastin preparations to the IRP. By definition, the ISI of the first IRP was 1.0. An additional term, the INR, was introduced to compare a given prothrombin ratio measurement to the IRP. Commercial vendors of thromboplastin preparations supply the ISI with each reagent lot. If the ISI is known, the INR for each clotting time is easily calculated. However, the ISI can be affected by instrumentation and other laboratory factors and thus must be verified by each testing site according to standards of the College of American Pathologists. Unfortunately, even with the INR, current prothrombin reagent/instrument calibration techniques are insufficient to provide good intralaboratory agreement.[51,52]

ACTIVATED PARTIAL THROMBOPLASTIN TIME

The partial thromboplastin time (PTT) is the clotting time obtained when "partial thromboplastin" is added to plasma. Partial thromboplastin is the phospholipid fraction of a tissue extract, and differs from a complete tissue extract (i.e., "thromboplastin") by the lack of tissue factor. The PTT is sensitive

to the intrinsic pathway of coagulation, but is most sensitive to the contact factors (i.e., factor XII, prekallikrein, high molecular weight kininogen) when a particulate "activating agent" (i.e., silica, celite, kaolin, micronized silica, ellagic acid) is added to the reaction (activated PTT, aPTT). Many different phosophlipid reagents animal and plant origin, such as cephalin, have been used as partial thromboplastins, and a variety of activating substances are in use[53].In the aPTT an aliquot of undiluted, platelet-poor plasma is incubated at 37^{o}C with an activator and phospholipid (partial thromboplastin). $CaCl_2$ is then added, and the time required for clot formation is measured by one of a variety of techniques (photo optical, electromechanical, etc.). The aPTT result is reported as the time required for clot formation after the addition of $CaCl_2$. The aPTT is functional determination of the intrinsic (factors XII, XI, IX, VIII, V, II,I,) and common pathways of coagulation.[54,55].The aPTT is utilized to detect congenital and acquired abnormalities of the intrinsic coagulation pathway, monitor patients receiving heparin or coagulation factor replacement therapy, and to detect inhibitors of the intrinsic and common pathways.[56].The aPTT clotting time may be influenced by many pre-analytical and analytical variables and caution must be used in the interpretation of the result. Preanalytical variables include slow or difficult specimen collection, an improper blood:anticoagulant ratio, failure to promptly mix the blood with the citrate anticoagulant, improper transport or storage, or a prolonged interval between specimen collection and analysis. The sensitivity of the assay to factor deficiencies, inhibitors, and

heparin also varies with the reagents used in the assay. Because of these variables, a normal aPTT result does not exclude a mild coagulation factor deficiency or the presence of a low titre or slow-reacting inhibitor. However, a significant prolongation of the aPTT indicates the presence of a factor deficiency (VIII, IX, XI, XII, prekallikrein, HMWK), while prolongation of both the PT and aPTT suggests a deficiency of factor I, II, V, or X. The aPTT is not affected by deficiencies of factor VII or XIII. Numerous modifications of the aPTT have been described for the functional analysis of specific coagulation-related substances which include the reptilase time, the Bethesda assay, protein C and protein S activity, and several assays for lupus anticoagulants (dilute Russell viper venom time[dRVVT], platelet neutralization test, and hexagonal phospholipid assay.

Specific anti-factor VIII antibodies (inhibitors) are a serious medical problem for patients with hemophilia. Mixing studies can detect the presence of inhibitors, but other assays are required for the precise measurement of antibody activity necessary for patient care.[57] The Bethesda assay is a modified aPTT based on the ability of factor VIII inhibitors to neutralize factor VIII activity in normal plasma. A series of dilutions of patient plasma are added to a standard amount of normal plasma and assayed for factor VIII levels after two hours incubation at 37^0C: the titre at which half of the FVIII activity remains is used to calculate the "Betheda units" of inhibition. Several modifications of the Bethesda assay have been developed to improve its sensitivity.[58-60]The new

Oxford assay is similar, but uses factor VIII concentrate as the source of factor VIII. Enzyme immunoassay, gel techniques, and other methods have been also used to detect inhibitors.

PLASMA MIXING STUDIES (Clotting Factor Inhibitor Screen, Circulating Anticoagulant Screen)

A prolonged clotting test (i.e., PT, aPTT, and/or thrombin time) indicates the presence of a factor deficiency or inhibitor of coagulation. The plasma mixing study is the initial step in the evaluation of a prolonged clotting time. The goal of a mixing study is to determine if the prolonged clotting time is shortened or "corrected" by mixing the test plasma with equal volume of normal pooled plasma (NPP; also called citrated normal plasma, CNP). Even a profound deficiency of a clotting factor, such as the 1% factor VIII level encountered in severe hemophilia, will be corrected to the normal range by mixing with NPP, since a 50% level of any factor will still yield a normal clotting time. "Factor assays" are then performed to identify the deficient clotting factor. The failure of a prolonged clotting test to correct in the mixing study indicates the presence of a "inhibitory" substance that is preventing clotting from occurring. Unfortunately, this is somewhat difficult to accomplish since there are several different types of inhibitors (also called "circulating anticoagulants"). "Specific inhibitors" are immunoglobulins with specificity for phospholipid ("lupus anticoagulants") or a specific clotting factor ("factor inhibitors"). "Global" or "non-specific" inhibitors affect more than part of the

clotting process and include fibrin(ogen) degradation products, some pathologic antibodies such as monoclonal paraproteins, and drugs such as heparin. Clinical and other laboratory clues are necessary to identify the inhibitor. For example, lupus anticoagulants are usually not associated with clinical bleeding, while specific factor inhibitors frequently cause bleeding. Generally, factor deficiencies produce a complete correction of the prolonged clotting time (i.e., corrected to within the normal range), specific antibodies show very little, if any correction, and non-specific may show a "partial correction," (i.e.,shortened clotting time but not to within the normal range). The presence of heparin and other nonspecific inhibitors can be confirmed by other coagulation tests such as the thrombin clotting time and reptilase time, while lupus anticoagulants are identified by a phospholipid-sensitive test such as the dilute Russell viper Venom time (dRVVT). The last clue is provided by the effect of incubation on the activity of the inhibitor. An "immediate" mixing study is performed by mixing equal amounts of the "test" plasma with NPP (1:1 mix) and immediately performing a clotting time(i.e., PT, aPTT, or TT) on the mixed plasma specimen.[61-63] Most factor inhibitors (except factor VIII) and a most lupus anticoagulants ("fast reacting inhibitors") produce an immediate clotting time inhibition and do not require incubation. In contrast, most factor VIII inhibitors and some lupus anticoagulants (15%) are weak and/or time dependent("slow reacting inhibitors"), and require incubation of the 1:1 plasma mixture at room temperature or 37^{o}C for one or two hours ("incubated mix") to cause

prolongation of the clotting time.[64] A false diagnosis of a factor deficiency can result without incubation, since slow-reacting inhibitors may correct the immediate mix. The markedly prolonged aPTT of coagulation factors, V and VIII, during incubated studies. Common criteria for correction of the patient sample include to within 5 seconds, or to within 10% or 15% of the NPP value.

PLASMA FIBRINOGEN

Fibrinogen is the most abundant clotting protein in the plasma, with a normal plasma level ranging from 200-400 mg/dl. The quantitative determination of plasma fibrinogen is essential in the diagnosis and management of many coagulopathies. In addition, since plasma fibrinogen levels are increased in some patients who develop myocardial infarction and stroke, there is interest in the measurement of fibrinogen for thrombotic risk assessment.[65] The washed clot method (total clottable fibrinogen assay,World Health Organization method) is the reference technique for fibrinogen determination. In this technique, citrated plasma is incubated for an extended period of time with thrombin in the presence of epsilon aminocaproic acid (EACA) to prevent digestion of the fibrin clot by plasmin. Other serum proteins are removed by washing, the clot is dissolved in concentrated urea, and the fibrinogen concentration is measured colorimetrically.[66] This technique is unsuitable for the determination of the large number of specimens encountered in the clinical laboratory, but, unfortunately, the accurate and precise measurement of fibrinogen with the automated coagulometer has proven

difficult. Immunoassays are rarely used. In spite of their flaws, the Von Clauss technique and the Clotting Rate Assay (Kinetic Fibrinogen Assay) are most widely used in the clinical laboratories. The Von Clauss technique is based upon the principle that when a high concentration of thrombin is added to plasma diluted in buffer (1:5 or 1:10), the effects of clotting inhibitors are diminished and the clotting time is directly proportional to the level of clottable fibrinogen.[67] Clotting times of patient plasma are read on a standard curve made with purified fibrinogen of known concentration to interpolate a fibrinogen level in the patient. The assay is accurate in the range of approximately 50 – 800 mg/dL. Since the von Clauss technique requires a high level of technical skill, a more recent prothrombin time-based kinetic assay is preferred by many laboratories. In this assay, the rate of increase in plasma turbidity is measured at 450 nm during the thrombin-catalyzed conversion of fibrinogen to fibrin.[68] This kinetic assay is rapid, economical, and can be fully automated.[69] Many studies have shown that fibrin degradation products cause an overestimation of the fibrinogen level by the washed clot and immunologic assays, and an underestimation by the clot-based techniques.[65]

WHO clinical staging of HIV disease in adults and adolescents

Clinical stage I

Asymptomatic

Persistent generalized lymphadenopathy

Performance scale 1:asymptomatic,normal activity

Clinical stage II

Moderate unexplained weight loss (under 10% of presumed or measured body weight)

Recurrent respiratory tract infections (sinusitis, tonsillitis, otitis media, pharyngitis)

Herpes zoster

Angular cheilitis

Recurrent oral ulcerations

Papular pruritic eruptions

Seborrhoeic dermatitis

Fungal nail infections

Performance scale 2: Symptomatic, normal activity.

Clinical stage III

Unexplained severe weight loss (over 10% of presumed or measured body weight)

Unexplained chronic diarrhoea for longer than 1 month

Unexplained persistent fever (intermittent or constant for longer than 1 month)

Persistent oral candidiasis

Oral hairy leukoplakia

Pulmonary tuberculosis

Severe bacterial infections (e.g. pneumonia, empyema, meningitis, pyomyositis, bone or joint infection, bacteremia, severe pelvic inflammatory disease)

Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis

Unexplained anaemia (below 8 g/dl), neutropenia (below 0.5 x 10^9/l) and/or chronic thrombocytopenia (below 50 x 10^9/l**)**

Performance Scale 3: bed ridden for <50% of day in last one month.

Clinical stage IV

HIV wasting syndrome

Pneumocystis jiroveci pneumonia

Recurrent severe bacterial pneumonia

Chronic herpes simplex infection (orolabial, genital or anorectal of more than 1 month's duration or visceral at any site)

Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)

Extrapulmonary tuberculosis

Kaposi sarcoma

Cytomegalovirus disease (retinitis or infection of other organs, excluding liver, spleen and lymph nodes)

Central nervous system toxoplasmosis

HIV encephalopathy

Extrapulmonary cryptococcosis including meningitis

Disseminated nontuberculous mycobacteria infection

Progressive multifocal leukoencephalopathy

Chronic cryptosporidiosis

Chronic isosporiasis

Disseminated mycosis (histoplasmosis, coccidiomycosis)

Recurrent septicaemia (including nontyphoidal *Salmonella*)

Lymphoma (cerebral or B cell non-Hodgkin)

Invasive cervical carcinoma

Atypical disseminated leishmaniasis

Symptomatic HIV-associated nephropathy or HIV-associated cardiomyopathy

Performance scale 4: Bed ridden for >50% of day in last one month.
(Source: Revised WHO clinical staging and immunological classification of HIV and case definition of HIV for surveillance 2006**)**

STATISTICAL ANALYSIS

Statistical analysis was carried out for 100 PLHAs after categorizing each variable- Age , sex ,duration of HIV, ART intake, platelet count, PT, aPTT, fibrinogen, LDH, CD4 count and clinical staging. Datas were analysed using Statistical package- SPSS software version 11.5.The values are presented as mean, standard error of mean, standard deviation, standard error of mean and median. Percentages were used to describe the proportions of discrete variables. The significance of difference between the proportions was indicated by the chi-square (x2) statistic. The significance of difference in mean between the groups was calculated by student t-test. Variables were considered to be significant if $P<0.05$. Intervariate analysis was done by using Pearson's r- value correlation.

OBSERVATIONS

100 PLHAs were included in the study irrespective of their ART status. They were investigated for platelet count, prothrombin time, activated partial thromboplastin time, mixing studies(if required),plasma fibrinogen, serum lactate dehydrogenase,CD4 count and some special investigations like lupus anticoagulant, bone marrow studies, D-Dimer were done when required. The observations of the study are as noted below:

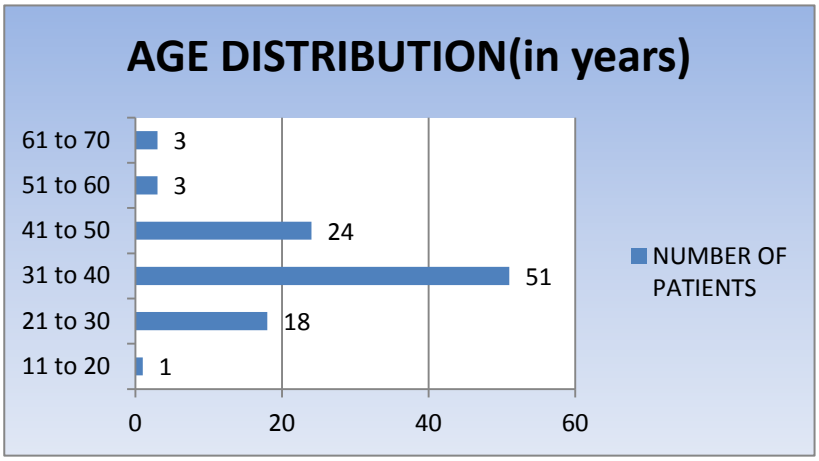
AGE DISTRIBUTION

Table no.1

AGE GROUP (years)	n	%
11 to 20	1	1
21 to 30	18	18
31 to 40	51	51
41 to 50	24	24
51 to 60	3	3
61 to 70	3	3

The mean age was found to be
37.96 years ± 8.795.

Chart showing age distribution



SEX DISTRIBUTION

Table no.2

AGE GROUP (years)	n		TOTAL
	MALE	FEMALE	
11 to 20	1	0	1
21 to 30	7	11	18
31 to 40	32	19	51
41 to 50	16	8	24
51 to 60	2	1	3
61 to 70	3	0	3
Total	61	39	100

DURATION OF HIV

No. of newly detected HIV patients(duration-0 months)-34

Mean HIV duration was 22.16 months ± 30.923

75% patients had HIV duration <25 months

Table no.3

	n
PRE-ART	50
ART	50

MODE OF TRANSMISSION:

Table no.4

	n
SEXUAL	99
VERTICAL	1

CLINICAL FEATURES AT PRESENTATION

Table no.5

SIGNIFICANT COMORBID ILLNESS	n
TB-MENINGITIS,LN,PT,MILIARY TB	10
CNS-STROKE,GBS,MYELOPATHY	12
LIVER DISEASE	7
RS-LRI,PNEUMOTHORAX	5
GIT-DIARRHEA,SPLEEN,PANCREAS INV.	12
FEVER,SEPSIS,CELLULITIS	5
VENOUS THROMBOSIS	2
MALIGNANCY	1
OTHERS-CARDIAC, RENAL, etc	6
NONE	40

Most of the patients (60%) had significant co-morbid illness at presentation. GIT and CNS manifestations were the common presentations in our study.

Chart showing the distribution of significant comorbid illnesses

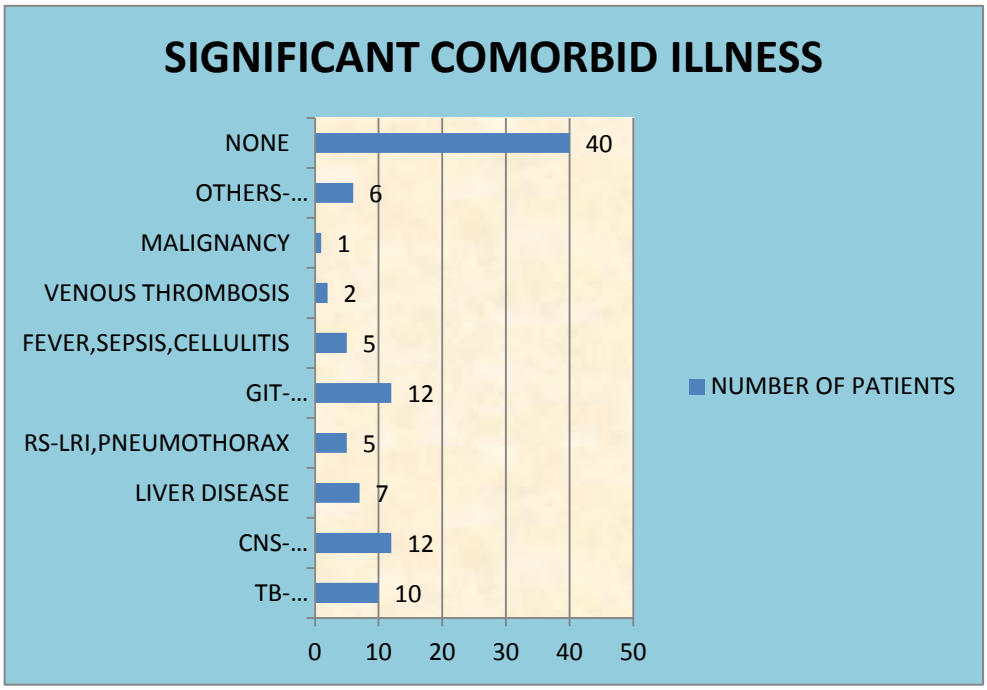


Table no.6

	Present
THROMBOSIS	8
BLEEDING	1

Among the hemostatic manifestations, thrombosis was much more frequent than bleeding. Infact, bleeding in the form of hematochezia occurred only in one patient and in that case also, cause was haemorrhoids rather than due to altered hemostasis.

Thrombotic manifestations in the form of six cases of ischemic stroke, one deep vein thrombosis and one cavernous sinus thrombosis.

Table no.7

	Pre-ART		On ART		Total
	n	%	n	%	N
Thrombosis	7	87.5	1	12.5	8

COINFECTION WITH VIRAL HEPATITIS

Table no.8

	n
HbsAg +ve	4
AntiHCV +ve	2

CD4 COUNT DISTRIBUTION

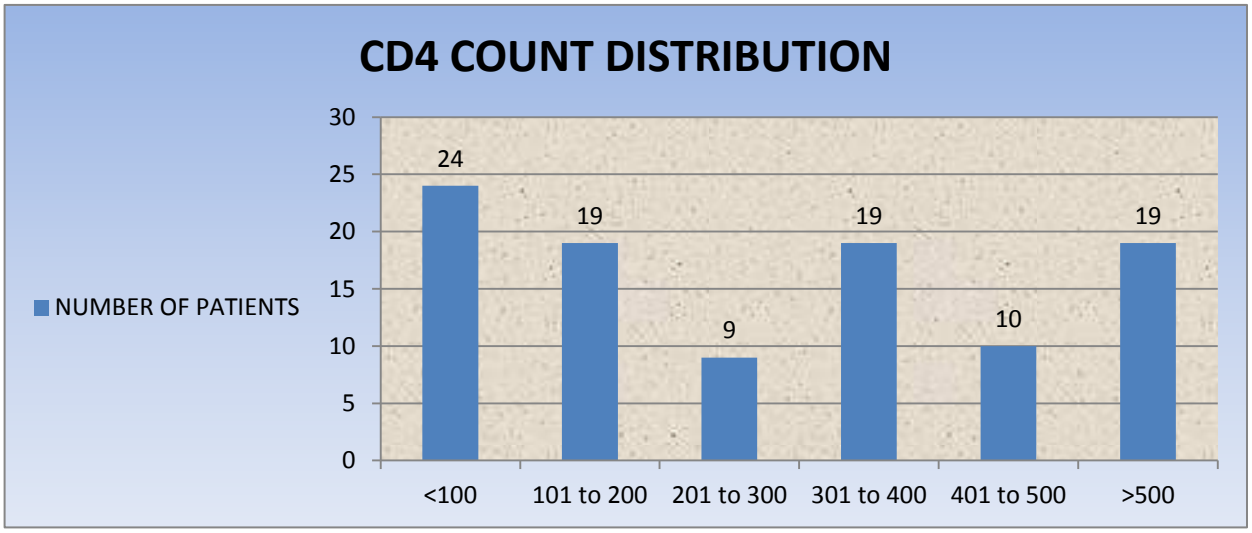
Table no.9

CD4 COUNT(cells/mm3)	n	%
<100	24	24
101 to 200	19	19
201 to 300	9	9
301 to 400	19	19
401 to 500	10	10
>500	19	19

The mean CD4 count in our study was 306.69 cells/mm^3

And the median was 267.Most of our patients (43%) had CD4 count < 200.

Chart showing the distribution of CD4 count



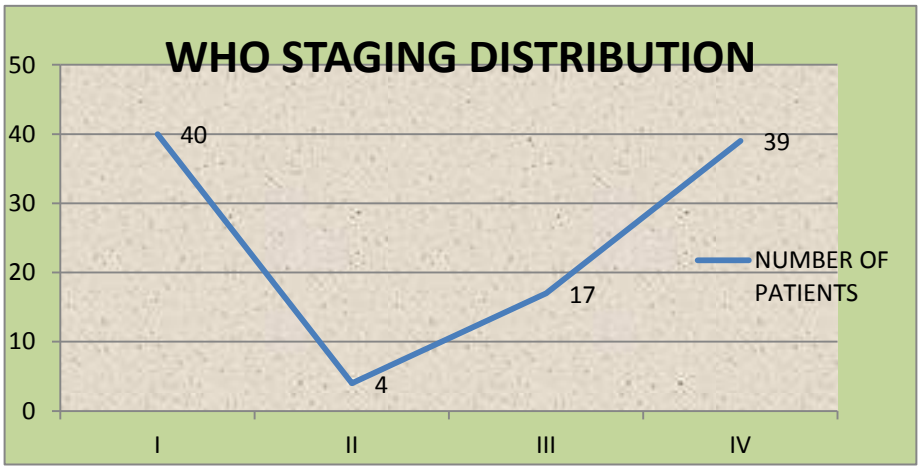
WHO STAGING DISTRIBUTION

Table no.10

WHO CLINICAL STAGING	n	%
I	40	40%
II	4	4%
III	17	17%
IV	39	39%

Majority of our patients were in extremes-stage I & IV as we had equal number of out-patients (most of whom were asymptomatic belonging to stage I) and in-patients(who had significant comorbid illnesses and AIDS defining conditions).

Chart showing the distribution of WHO staging



PLATELET COUNT DISTRIBUTION

Table no.11

PLATELET COUNT(lakh cells/mm3)	n	%
<1	10	10%
1 to 1.5	41	41%
1.51 to 2.0	31	31%
>2	18	18%

The mean platelet count was 1.64 lakhs/mm^3 $\pm$ 0.8431 with the majority falling in the range of 1-1.5lakhs/mm^3.Median was 1.50 lakhs/mm^3. Thrombocytopenia($\leq$1.5 lakhs/mm^3) was found in 51%.

Chart showing the distribution of platelet count

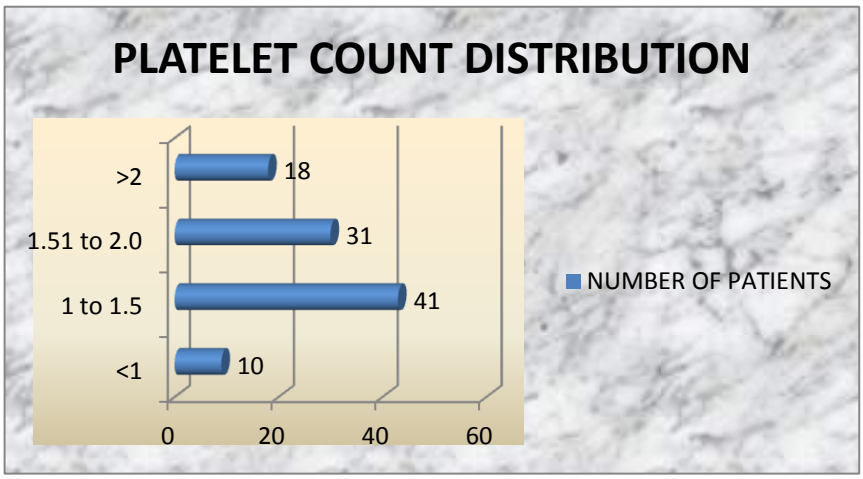


Table no.12

Thrombocytopenia	n	%
Mild (1-1.5lakhs)	41	41
Moderate (0.5-1lakh)	9	9
Severe (<50,000)	1	1

THROMBOCYTOPENIA vs WHO STAGING

Table no.13

THROMBOCYTOPENIA	WHO STAGES			
	I	II	III	IV
Mild (1-1.5lakhs)	17(41.46%)	2(4.87%)	9(21.95%)	13(31.7%)
Moderate (0.5-1lakh)	-	2(22.23%)	1(11.12%)	6(66.67%)
Severe (<50,000)	-	-	-	1(100%)

In our study, majority of thrombocytopenia noted in stages I & IV. There was no statistical significance between thrombocytopenia and WHO staging (p value-0.066).

THROMBOCYTOPENIA vs CD4 COUNT

Table no.14

CD4 COUNT(cells/mm3)	THROMBOCYTOPENIA		
	Mild (1-1.5lakhs)	Moderate (0.5-1lakh)	Severe (<0.5lakh)
< 100	9(21.95%)	4(44.44%)	1(100%)
101 to 200	5(12.19%)	1(11.11%)	-
201 to 300	4(9.75%)	-	-
301 to 400	11(26.82%)	2(22.22%)	-
401 to 500	4(9.75%)	-	-
>500	8(19.51%)	2(22.22%)	-

Mild cases of thrombocytopenia were distributed evenly in all ranges of CD4 count, but still almost 66% cases were reported in patients with CD4 count >200 cells/mm^3.On the other hand, moderate and severe thrombocytopenia were noted in patients with CD4 count <200cells/mm^3.There was no statistical significance between CD4 count and thrombocytopenia (p value-0.663).

THROMBOCYTOPENIA vs COMORBID ILLNESS

Table no.15

THROMBOCYTOPENIA	SIGNIFICANT COMORBID ILLNESS	
	PRESENT	ABSENT
Mild(1-1.5 lakhs)	24(58.53%)	17(41.46%)
Moderate(0.5-1 lakh)	9(100%)	-
Severe(<50,000)	1(100%)	-

Majority of patients with thrombocytopenia had significant comorbid illness at presentation. But still mild thrombocytopenia could be noted among PLHAs without significant illness. There was no statistical significance between thrombocytopenia and presence of comorbid illness (p value-0.135).

THROMBOCYTOPENIA vs AGE DISTRIBUTION

Table no.16

AGE GROUP (years)	THROMBOCYTOPENIA			n	%
	Mild	Moderate	Severe		
11 to 20	-	1	-	1	1.96
21 to 30	8	2	-	10	19.60
31 to 40	20	5	1	26	50.98
41 to 50	10	-	-	10	19.60
51 to 60	1	1	-	2	3.92
61 to 70	2	-	-	2	3.92

PROTHROMBIN TIME:

Table no.17

PROTHROMBIN TIME	n	%
Normal	48	48
Prolonged	48	48
Reduced	4	4

Mean PT was 13.52 seconds $\pm$ 2.347.Median was 13 seconds.

PROTHROMBIN TIME vs WHO STAGING

Table no.18

PROTHROMBIN TIME	WHO STAGING			
	I	II	III	IV
Normal	26	1	9	12
Prolonged	11(22.91%)	2(4.16%)	9(18.75%)	26(54.16%)
Reduced	3	1	-	-

Majority of prolonged PT was noted in patients with stage IV disease. There was statistical significance for PT with WHO staging (p value-0.015) by Chi square test.

PROTHROMBIN TIME vs CD4 COUNT

Table no.19

CD4 COUNT(cells/mm3)	PROTHROMBIN TIME		
	Normal	Prolonged	Reduced
< 100	10	14(29.16%)	-
101 to 200	12	7(14.58%)	-
201 to 300	5	4(8.33%)	-
301 to 400	9	9(18.75%)	1
401 to 500	5	3(6.25%)	2
>500	7	11(22.91%)	1

There was no statistical significance between CD4 count and PT (p value-0.114) by Chi square test.

PROTHROMBIN TIME vs COMORBID ILLNESS

Table no.20

PROTHROMBIN TIME	SIGNIFICANT COMORBID ILLNESS	
	PRESENT	ABSENT
Normal	22	26
Prolonged	37(77.08%)	11(22.91%)
Reduced	2	-

Majority of patients with prolonged PT (77%) had significant comorbid illness. There was statistical significance between PT and co-morbid illness (p value-0.006) by Chi square test.

ACTIVATED PARTIAL THROMBOPLASTIN TIME

Table no.21

ACTIVATED PARTIAL THROMBOPLASTIN TIME	n	%
Normal	72	72%
Prolonged	22	22%
Reduced	6	6%

Mean was 34.978 seconds ± 10.153.Median was 32 seconds.

aPTT vs WHO STAGING

Table no.22

aPTT	WHO STAGING			
	I	II	III	IV
Normal	35	3	12	22
Prolonged	1(4.54%)	1(4.54%)	6(27.27%)	14(63.63%)
Reduced	4	-	-	2

Majority of patients with prolonged aPTT were noted in advanced stages III and IV disease. There was statistical significance for aPTT with WHO staging(p value-0.008).

aPTT vs CD4 COUNT

Table no.23

CD4 COUNT(cells/mm3)	aPTT		
	Normal	Prolonged	Reduced
< 100	17	7(31.81%)	-
101 to 200	17	2(9.09%)	-
201 to 300	7	2(9.09%)	-
301 to 400	12	5(22.72%)	2
401 to 500	6	2(9.09%)	2
>500	13	4(18.18%)	2

There was no statistical significance between CD4 count and aPTT (p value-0.339) by Chi square test.

aPTT vs COMORBID ILLNESS

Table no.24

ACTIVATED PARTIAL THROMBOPLASTIN TIME	SIGNIFICANT COMORBID ILLNESS	
	PRESENT	ABSENT
Normal	37	35
Prolonged	21(95.45%)	1(4.55%)
Reduced	3	3

Almost all except one patient with prolonged aPTT had significant comorbid illness. There was statistical significance between aPTT and comorbid illness (p value-0.001) by Chi square test.

PLASMA FIBRINOGEN:

Table no.25

PLASMA FIBRINOGEN(mg/dl)	n	%
<180	36	36
180-350	60	60
>350	4	4

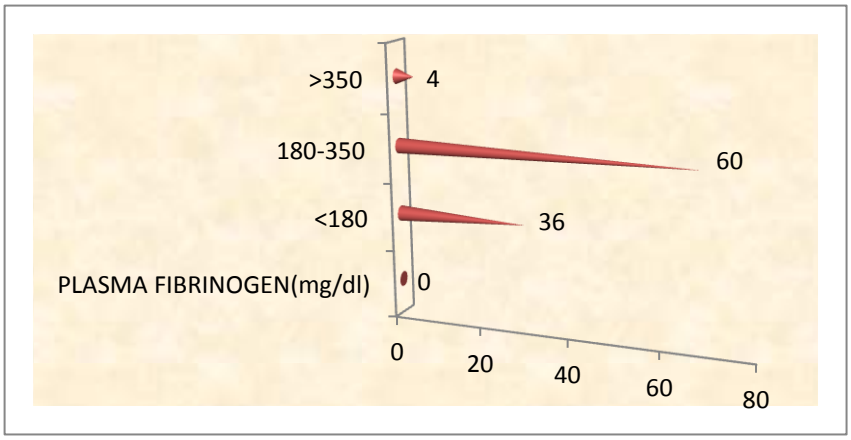
Our lab normal plasma fibrinogen level-180 to 350 mg/dl.

Most of our patients (60%) had normal fibrinogen level in plasma.

Mean was 198.27 ± 70.295.Median was 193.

Level was decreased in 36 cases and increased in 4.

Chart showing the distribution of plasma fibrinogen



FIBRINOGEN vs WHO STAGING

Table no.26

FIBRINOGEN	WHO STAGING			
	I	II	III	IV
Normal	25	2	8	16
Increased	-	-	1(25%)	3(75%)
Reduced	7(19.44%)	2(5.55%)	9(25%)	18(50%)

Majority of fibrinogen derangements are noted among advanced stages. There was statistical significance between fibrinogen and WHO staging (p value-0.018) by Chi square test.

FIBRINOGEN vs CD4 COUNT

Table no.27

CD4 COUNT(cells/mm3)	FIBRINOGEN		
	Normal	Increased	Reduced
< 100	9	2(50%)	13(36.11%)
101 to 200	14	-	5(13.88%)
201 to 300	7	-	2(5.55%)
301 to 400	8	2(50%)	9(25%)
401 to 500	9	-	1(2.77%)
>500	13	-	6(16.66%)

Almost 50% of PLHAs with reduced fibrinogen level had CD4 count <200 cells/mm^3. There was no statistical significance between fibrinogen and CD4 count (p value-0.072) by Chi square test.

FIBRINOGEN vs COMORBID ILLNESS

Table no.28

FIBRINOGEN	SIGNIFICANT COMORBID ILLNESS	
	PRESENT	ABSENT
Normal	28	27
Increased	4(100%)	-
Reduced	29(80.55%)	7(19.44%)

Majority of patients with fibrinogen derangements had significant comorbid illness. There was statistical significance between fibrinogen and comorbid illness (p value-0.001) by Chi square test.

SERUM LACTATE DEHYDROGENASE

Table no. 29

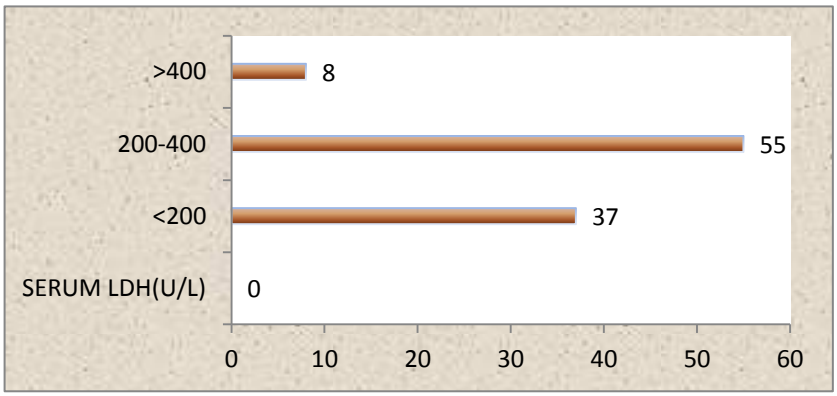
SERUM LDH(U/L)	N	%
<200	37	37
200-400	55	55
>400	8	8

Our lab normal reference range for LDH was 200-400U/L.

Mean was 257.12 $\pm$ 128.25.Median was 223U/L.

Majority had normal LDH (55%) or reduced LDH (37%).

Chart showing the distribution of serum LDH



LDH vs WHO STAGING

Table no.30

LDH(U/L)	WHO STAGING			
	I	II	III	IV
Normal	21	2	11	21
Increased	-	1(12.5%)	1(12.5%)	6(75%)
Reduced	19	1	6	11

About 75% of patients with raised LDH belonged to stage IV. There was statistical significance between WHO staging and LDH(p value-0.005) by student t test.

LDH vs CD4 COUNT

Table no.31

CD4 COUNT(cells/mm3)	LDH		
	Normal	Increased	Reduced
< 100	16	3(37.5%)	5
101 to 200	8	-	11
201 to 300	5	-	4
301 to 400	9	3(37.5%)	7
401 to 500	6	-	4
>500	11	2(25%)	6

LDH was distributed in CD4 count of all varied ranges. There was no statistical significance between LDH and CD4 count (p value-0.363) by Chi square test.

LDH vs COMORBID ILLNESS

Table no.32

SERUM LDH	SIGNIFICANT COMORBID ILLNESS	
	Present	Absent
Normal	35	18
Increased	8(100%)	-
Reduced	18	14

All patients with increased LDH had significant comorbid illness. There was statistical significance between LDH and comorbid illness (p value-0.022) by Chi square test.

STATISTICAL ANALYSIS

(student t test)

Analysis of hemostatic parameters between patients on ART & non-ART

	PRE-ART		ART		P value
	Mean	SD	Mean	SD	
Platelet	1.76	1.08	1.52	0.47	0.156
PT	13.93	2.68	13.1	1.89	0.077
aPTT	35.5	10.29	33.5	7.72	0.281
Fibrinogen	203.53	85.58	193.02	51.02	0.458
LDH	250.12	116.95	264.12	139.47	0.588

The above analysis shows that there is no statistical significance between hemostatic parameters of patients on ART & without ART.

Analysis of hemostatic parameters with WHO staging

Parameters	STAGE I		STAGE II		STAGE III		STAGE IV		P value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Platelet	1.67	0.44	0.97	0.28	1.62	0.64	1.67	1.2	0.455
PT	12.39	1.33	12.27	1.7	14.2	2.07	14.49	2.8	0.000
aPTT	29.67	4.4	33.42	4.28	38.08	10.09	38.14	10.48	0.000
LDH	219.3	46.17	422.2	361.8	241.8	99.45	286.8	145.2	0.005

From the above analysis, it is shown that there is statistical significance of PT, aPTT, LDH with WHO staging.

Analysis of hemostatic parameters with CD4 count

Parameters	CD4 count P value
Platelet	1
PT	0.257
aPTT	0.194
Fibrinogen	0.291
LDH	0.233

The above analysis shows no statistical significance between hemostatic parameters and CD4 count.

Analysis of hemostatic parameters with ART duration

Parameters	ART duration P value
Platelet	0.853
PT	0.369
aPTT	0.225
Fibrinogen	0.370
LDH	0.712

There was no statistical significance between hemostatic parameters and ART duration.

CORRELATIONS

(Pearson method)

Hemostatic parameters vs ART duration

Parameters	ART duration	
	Pearson correlation coefficient	P value
Platelet	0.015	0.920
PT	0.124	0.391
aPTT	-0.055	0.706
Fibrinogen	-0.060	0.681
LDH	-0.053	0.717

Hemostatic parameters vs HIV duration

Parameters	HIV duration	
	Pearson correlation coefficient	P value
Platelet	-0.148	0.141
PT	-0.026	0.797
aPTT	-0.062	0.539
Fibrinogen	-0.174	0.083
LDH	0.173	0.085

Hemostatic parmeters vs CD4 count

Parameters	CD4 count	
	Pearson correlation coefficient	P value
Platelet	0.049	0.630
PT	-0.137	0.174
aPTT	-0.069	0.497
Fibrinogen	0.000	0.997
LDH	0.091	0.368

From the above correlations analysis, there was no correlation found between hemostatic parameters and HIV duration, ART duration and CD4 count.

DISCUSSION

It is well appreciated that PLHA's irrespective of their ART status presents with many systemic disturbances including hemostatic derangements. However, not all these abnormalities are reflected clinically. Studies pertaining to hemostatic changes in PLHA's are only few throughout the world and in India, there was only one study[73] (***BMC Blood Disorders*** **2009, 9:5)** which included coagulation parameters also as a part of haematological manifestations. The observation made in the PLHAs with respect to platelet count, prothrombin time, activated partial thromboplastin time, plasma fibrinogen, serum lactate dehydrogenase, CD4 count and WHO stages were analysed and the following inferences were drawn.

AGE DISTRIBUTION

The mean age of the study population was 37.96 years±8.795. About 51% of them were in age group of 30-40 years. As per the data released by Tamil Nadu State AIDS control society (TANSACS) about 50% of HIV infected patients belonged to 30 - 49 years at the time of diagnosis [(70)].In the western countries about 36% of them were in the 35-44 years age group at the time of diagnosis, which happens to be the major age group affected [(71)].Our data is in accordance with these data.

SEX DISTRIBUTION

In our study population,61% were males and 39% were females. The male to female ratio was approximately 3:2 The sex ratio of PLHA is at the time of diagnosis was 8:3 in India[70] and 7:3 in western countries[71].The increased female representation in our study is probably a more disciplined followup observed among women in the outpatient clinic.

WHO STAGE DISTRIBUTION

Most of the PLHA's were in extremes in stages I (40%) and IV (39%).This is because our study comprised equal number of inpatients(who were admitted in ward with significant comorbid illnesses) and out patients(mostly asymptomatic attending ART op).

CD4 COUNT DISTRIBUTION

In our study, the mean CD4 count was 306.69 cells/mm^3 $\pm$237.95 and median was 267 cells/mm^3.It ranged from 21 to 1247 cells/mm^3. Since our study comprised of PLHA's in extremes of stages, we had equal distribution of patients in CD4 counts of varied ranges. 43% of PLHA's in our study had CD4 count<200 cells/mm^3 and the rest had CD4 count >200 cells/mm^3.

THROMBOCYTOPENIA

Mean platelet count in our study was 1.64 $\pm$0.84 lakhs/mm^3 and median was 1.50 lakhs/mm^3.It ranged from 30,000 to 7.42 lakhs/mm^3.Thrombocytopenia (<1.5 lakhs/mm^3) was found in 51% of PLHA's in our study.

Mild thrombocytopenia(1-1.5 lakhs/mm^3)was found in 41 PLHA's ,majority of whom were in either stage I(41%) or in stage IV(31%).They were distributed in CD4 count of all varied ranges. Majority of them had significant co-morbid illness(58.5%) in the form of viral or alcoholic hepatitis, acute febrile illnesses and infections(pulmonary or meningeal TB, splenic candidiasis, splenic abscess, sepsis and cellulitis). However few were asymptomatic (41.46%) falling under stage I which signifies the varied mechanisms attributed to HIV related thrombocytopenia and its early occurrence in the course[10] .

Among PLHA's with **moderate and severe thrombocytopenia**, most (67%) of them belonged to advanced stage (stage IV).CD4 count didn't correlate with the severity of thrombocytopenia. All had significant co-morbid illness in the form of stroke, infections(TB, Cryptococcal meningitis, sepsis) and viral hepatitis. Four patients with moderate to severe thrombocytopenia had thrombotic manifestations-three with ischemic stroke and one with cavernous sinus thrombosis. None had bleeding manifestations.

West Indian Med J. 2009 Nov; 58(5):437-40 concluded that among the HIV-infected patients, platelet count did not differ significantly ($p > 0.05$) between those with CD4 count < 200 cells//L and those with CD4 count>200 cells/microL. In our study also, there was neither statistical significance (P value=1) nor correlation(P value=0.63) between platelet count and CD4 count.[72]

Two previous Indian studies-***BMC Blood Disorders* 2009, 9:5 & *Turk J Hematol* 2008, 25:13-9** also found no significant correlation between thrombocytopenia and CD4 count.[73,74]

PROTHROMBIN TIME

Prothrombin time was prolonged in 48% of our study population with the mean PT of 13.52±2.347 seconds. Median was 13 seconds. Majority of patients with prolonged PT belonged to advanced stage IV (54%).There was statistical significance between PT and WHO staging (P value-0.000).However, the CD4 count didn't correlate well as PT was prolonged in PLHA's with CD4 count of all ranges. Majority were males (72.91%) suggesting the possible role of alcoholism deranging the PT. About 77% of PLHA's with prolonged PT had significant co-morbid illness in the form of liver disease, pancreatitis, diarrheal diseases, acute febrile illnesses, infections(TB, Candidiasis, Cryptococci, sepsis), stroke, venous thrombosis and malignancy(carcinoma cervix). All the patients with viral co-infection had prolonged PT(4 were HbsAg+ and 2 were anti-HCV+). However 11 patients with prolonged PT didn't have any

significant illness at presentation and they all were grouped under stage I. This signifies that PT prolongation can occur in any stage of HIV infection and usually asymptomatic. With mixing studies, most of the prolonged PT got corrected which reveals the possible clotting factor deficiencies in HIV infection and its associated co-morbid illnesses. Few patients in whom prolongation of PT was not corrected fully, was probably due to chronic subclinical consumption coagulopathy or acquired inhibitors.

ACTIVATED PARTIAL THROMBOPLASTIN TIME

Among the 100 PLHA's analysed, 22 had prolonged aPTT with the mean of 34.978 seconds±10.153. Median was 32 seconds. Majority of patients(63.63%) with prolongation of aPTT were in advanced stage IV. There was statistical significance between aPTT and WHO staging (P value=0.000).Except one, all had significant co-morbid illnesses in the form of liver disease, stroke, ARDS, febrile illnesses and infections(TB, Cryptococci, Candidiasis, sepsis). None had bleeding manifestations. Three patients with prolonged aPTT had thrombotic manifestations in the form of ischemic stroke. Among those 3 patients, one had associated HCV co-infection which might have contributed to hypercoagulability in addition to factors contributed by HIV infection.

PROLONGED PROTHROMBIN TIME & aPTT

Among the 100 PLHA's, 20 had prolongation of both PT and aPTT. Majority of patients were in advanced stages IV (65%) and III(30%). All(95%) except one had significant co-morbid illnesses in the form of liver disease, stroke, chronic diarrhea, febrile illnesses and infections(TB, Cryptococci, sepsis). None had bleeding manifestations. Two had thrombotic manifestations in the form of stroke. Five patients with prolonged PT and aPTT had viral co-infection(3 HbsAg +, 2 anti-HCV +).

West Indian med. j. vol.58 no.5 Mona Nov. 2009 published a study done by R Omoregie et al among HIV patients in Benin City, Nigeria which concluded that PT and APTT are higher in HIV patients with *CD4* count < 200 cells/µL, but only PT correlates with *CD4* count and the correlation was observed only in HIV patients with *CD4* count < 200 cells/µL. This study excluded symptomatic PLHA's. Endothelial activation is suggested as the cause of the coagulation defect.[72] But in our study done among Indian population there was no correlation of CD4 count with any of hemostatic parameters including PT as we included significant number of symptomatic patients.

In the study conducted in PGIMER, Chandigarh on **Profile of hematological abnormalities in Indian HIV infected individuals,** there was no coagulation abnormalities detected.[73] This was in contrast to our study done

among Tamil Nadu patients where much of derangements of coagulation profile could be noted.

PLASMA FIBRINOGEN

Among the 100 PLHA's analysed in our study, 40% had fibrinogen derangements out of normal range. 36 patients had reduced fibrinogen levels while 4 had raised fibrinogen level in plasma. Mean was 198.27±70.295 mg/dl. Median was 193mg/dl.19 of 36 patients with reduced fibrinogen were on ART. So, the significant number of patients with reduced fibrinogen level in our study probably reflects the use of NNRTIs as the first line therapy for newly diagnosed PLHAs. **Erin Madden et al[79] -AIDS 2008;22(6):707-715-** concluded that Protease inhibitor use is associated with elevated fibrinogen levels which may contribute to increased risk of atherosclerosis in HIV-infected patients. Conversely, NNRTI use is associated with lower fibrinogen levels which may decrease risk of atherosclerosis.

The increase in fibrinogen noted in 4 PLHAs could be attributed to its response as an acute phase reactant as all these patients had significant co-morbid illness. Most of the fibrinogen derangements had occurred in advanced stage IV. Almost 50% of our cases with reduced fibrinogen level had CD4 count <200 cells/mm^3. Among the PLHA's with reduced fibrinogen, about 81% had significant co-morbid illnesses in the form of stroke, liver disease,

malignancy (Ca cervix) and infections (TB, Candidiasis, Cryptococci, pneumonia, sepsis). Five patients with reduced fibrinogen had thrombotic manifestations. All the four patients with increased fibrinogen had significant co-morbid illnesses and two had stroke among them.

SERUM LACTATE DEHYDROGENASE

Only eight of our patients had raised LDH. Mean LDH for our 100 cases was 257.12±128.25 U/L. Median was 223U/L. About 75% of patients with raised LDH belonged to stage IV. All the patients with raised LDH had significant co-morbid illnesses in the form of TB, ARDS, Hepatitis and malignancy (Carcinoma cervix). There was statistical significance for LDH with WHO staging (P value=0.005).The increased LDH could be attributed to microangiopathic haemolysis in 2 cases (malignancy, RHD), hepatitis (alcoholic) in a case and hemophagocytic histiocytosis with pancytopenia in a young boy with vertically transmitted HIV.The haemophagocytosis was made detected in the bone marrow.

THROMBOTIC EVENTS

In our study population of 100 PLHA's irrespective of their ART status, we noted eight(8%) patients with thrombotic events in the form of arterial thrombosis(ischemic stroke) as well as venous thrombosis(deep venous thrombosis and cavernous sinus thrombosis).**Thrombosis and a Hypercoagulable State in HIV-Infected Patients-Yu-Min P. Shen et al** –also gave an incidence of thromboembolic events ranging from 0.26 to 7.6%.[75] All the eight patients were ambulatory and had no known risk factors for pathologic thrombus formation.

On analysis of their clinical as well as hemostatic profile, we made the following observations:

1) Mean age was 39.125±11.85 years. Median was 37.5 years. It ranged from 27 to 65 years. This shows that thrombotic events are common in younger PLHA's suggestive of a probable hypercoagulable state. This age group correlated with the article- **Stroke in HIV infection and AIDS- Dobbs and Berger-**which also determined the increased risk of stroke to be most apparent in the young HIV-infected population in whom other risk factors for stroke are seldom evident[76]. Mechanisms underlying the increased risk include opportunistic infectious meningitides and vasculitides, primary HIV vasculopathy, altered coagulation and cardioembolic events, although the cause may be multifactorial or remain cryptic.

2) Seven of them were males and one was female.

3) Mean duration of HIV was 6.5±10.88 months. Five of them were newly detected as positive for HIV serology with this presentation suggesting that thrombotic manifestation may be the initial presentation of HIV infection itself.

4) Only one was on ART for 12 months duration with first line regimen-zidovudine, lamivudine and nevirapine therapy.

5) The mode of transmission was heterosexual in all the patients.

6) None had bleeding manifestations.

7) Among the eight cases, six presented as ischemic stroke, one as deep venous thrombosis of upper limb and one as cavernous sinus thrombosis. On analysing the six cases of stroke, five presented as young stroke with age ≤40 years. Lipid profile of all the patients with stroke were within normal limits. One had positive serology for Hepatitis C virus and all others didn't have any co-morbid illnesses other than the modifiable risk factors like smoking and alcoholism.

8) Their renal and liver function tests were normal except the one with anti-HCV positivity who had raised bilirubin and liver enzymes.

9) Mean haemoglobin was 10.45±2.769 gms/dl. It ranged from 6.6 to 14.8 gms/dl with the median of 10.2.

10) Mean platelet count was 1.94±2.29lakhs/mm^3.Median was 1.23 with the range from 0.30 to 7.42 lakhs/mm^3. Four had thrombocytopenia of whom three presented as ischemic stroke and one had cavernous sinus thrombosis.

11) Two had isolated prolongation of prothrombin time and one with isolated prolongation of aPTT. Two others had prolongation of both PT and aPTT.

12) Among the eight PLHA's with thrombosis, seven had fibrinogen derangements: 5 with reduced level and 2 with increased fibrinogen level. Mean was 205.15±153.67 mg/dl. Median was 155.50 with the range from 57.2 to 484mg/dl. Among the six patients with stroke, 4 had reduced fibrinogen while 2 had increased level.

13) Mean LDH level was 231.5± 64.78 U/L. Median was 203 with the range from 163 to 314 U/L.

14) Mean CD4 count was 179.375±172.30 cells/mm^3.Median was 97 with the range from 85 to 563 cells/mm^3.Six had CD4 count <200cells/mm^3, suggesting the occurrence of thrombotic manifestations with severe immunosuppression.

15) All the eight patients belonged to WHO clinical stage IV.

16) Special investigations like D-Dimer and LAC in seven patients with thrombosis excluding that elderly male with stroke. LAC was positive only in one patient and D-Dimer was significantly elevated in 3 patients.

INTERESTING CASES

We analysed few interesting cases and did some relevant special investigations.

- A 36 yr old male PLHA on irregular ART presented with ischemic stroke & severe thrombocytopenia whose PT, aPTT was also prolonged which with mixing studies showed delayed type inhibitor.LAC was also done which turned to be positive. Bone marrow was normocellular with normal megakaryocytes and erythropoiesis.
- Marrow was also done for a young boy with vertically transmitted HIV who presented with pancytopenia. The marrow was hypocellular with dimorphic erythropoiesis and normal myeloid maturation. Megakaryocytes were adequate with hyperlobulated forms and megakaryocyte bare nuclei. Mild Lymphocytosis 6% with few atypical cells and increased iron stores(2+) with no sideroblasts seen. Hemophagocytosis+. Viral serology for hepatotropic viruses and TORCH infection were negative. So we attribute the pancytopenia to ART therapy(zidovudine induced with probable superadded viral infection).
- Another 27 yr old male who presented as young stroke had moderate thrombocytopenia with elevated fibrinogen, normal D-Dimer and negative LAC. Two other young PLHA's with ischemic stroke had reduced fibrinogen with normal platelet count and D-Dimer and negative

LAC. PT and aPTT were also normal in all these three patients. So this directs us towards the possible HIV associated vasculopathy. **Mu et al.: Update on HIV-associated Vascular Diseases**-analysed the profound functional alterations of the endothelium in HIV infection through various clinical and laboratory studies. The virus and its viral proteins such as gp120, Tat, and Nef are able to induce expression of several adhesion molecules and inflammatory cytokines such as ICAM-1,VCAM-1, E-selectin, TNF-alpha and IL-6. Leukocyte adherence to the endothelium is enhanced as the expression of these cell adhesion molecules increases. Elevated circulating levels of vWF, a glycoprotein facilitating platelet adhesion, synthesized in endothelial cells and inflammatory cells, are elevated and correlate to circulating levels of inflammatory cytokines. A hypercoagulable state is induced depending on plasma HIV load. In addition, HIV and its viral proteins can also induce endothelial apoptosis and increase endothelial permeability. These effects could significantly contribute to vascular disease formation. However, the molecular mechanisms underlying HIV associated vascular diseases and endothelial injury are not completely understood.[77]

- Another young patient with sepsis and cavernous sinus thrombosis had elevated D-Dimer with much reduced fibrinogen suggestive of DIC and he ultimately succumbed to his illness. Another patient with ischemic stroke and Hepatitis C Virus co-infection also had elevated D-Dimer with

much reduced fibrinogen and who also expired. Both the patients were not on ART. This proved D-Dimer elevation as an important marker for all cause mortality in PLHAs.[10] **Inflammatory and Coagulation Biomarkers and Mortality in Patients with HIV Infection-Lewis H. Kuller for the INSIGHT SMART Study Group** concluded that IL-6 and D-Dimer were strongly related to all-cause mortality. Interrupting ART may further increase the risk of death by raising IL-6 and D-Dimer levels. They also hypothesized that increased HIV-RNA levels following ART interruption induced activation of tissue factor pathways, thrombosis, and fibrinolysis. Therapies that reduce the inflammatory response to HIV and decrease IL-6 and D-Dimer levels may warrant investigation.[78]

SUMMARY AND CONCLUSION

Our study included 100 patients positive for HIV ELISA- 50 outpatients and 50 inpatients. 50 patients were on ART and 50 were pre-ART. The hemostatic parameters were evaluated in all individuals irrespective of their clinical symptoms.

Thrombocytopenia was noted among 51 PLHA's in our study but there was no statistically significant correlation with either CD4 count or WHO staging of disease. Significant comorbid illnesses were identified in all PLHA's with moderate-severe thrombocytopenia and 58.5% of PLHA's with mild thrombocytopenia. The thrombocytopenia in otherwise asymptomatic patients might be attributed to HIV infection itself or due to drugs.

Prothrombin time was prolonged in 48% patients in our study and aPTT in 22% of our patients. The higher incidence of PT prolongation could be attributed to alcoholic hepatic dysfunction. All patients with aPTT prolongation had significant comorbid illnesses. The increased coagulation parameters got corrected with normal plasma except the one who presented with stroke and found to be positive for LAC.

The most interesting feature noted in our study was the striking absence of bleeding diathesis despite the hemostatic derangements. But thrombotic manifestations were noted in eight patients in the form of ischemic stroke, DVT

and cavernous sinus thrombosis. This leads us to the possible influence of HIV infection & ART on the antithrombotic factors like protein C, protein S, anti-thrombin III and others which needs to be distinctly delineated. Most of the studies have shown significant changes in the presence of antiphospholipid antibodies and the lupus anticoagulant; deficiencies of protein C, protein S, heparin cofactor II, and antithrombin; and increased levels of von Willebrand factor and D-Dimer and these abnormalities correlate with the severity of HIV-associated immunosuppression as measured by CD4+ cell counts and the presence of infectious or neoplastic diseases associated with HIV infection.

The emphasis of our observation should also be on these lines, if effective prophylactic measures against thrombosis need to be incorporated in routine therapeutic strategies. This provides a strong rationale for careful prospective studies to evaluate the prevalence, pathogenesis, and risk factors associated with the development of thromboembolic complications in patients with HIV infection.

SCOPE FOR FURTHER STUDIES

Evaluation of i) platelet functional studies, ii) coagulation factor assays, and iii) antithrombotic factor assays in PLHAs before commencing ART and while on treatment should be done. This could decide on the need for including thromboprophylaxis on HIV patients irrespective of their ART status, should the clinical profile suggest either virus induced or drug induced predisposition to thrombosis.

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PROFORMA

PRESENT HISTORY

- Duration of HIV infection
- Duration of ART intake
- Bleeding manifestations

PAST & PERSONAL HISTORY

- Jaundice
- Alcoholism
- Blood Transfusion
- Premarital/Extramarital contact
- Homosexuality
- Tattooing/IV drug abuse
- Native treatment
- Drug intake like ATT/Anti-epileptics
- Surgeries in past

In women-obstetric and menstrual history

CLINICAL EXAMINATION

- Anaemia
- Jaundice
- Pedal Oedema
- Stigmata of Tuberculosis
- Lymphadenopathy
- Clubbing
- Purpura, bleeding gums
- Oral candidiasis
- Glossitis
- Herpes zoster
- Weight

VITAL SIGNS

- Pulse rate
- Blood Pressure
- Respiratory rate
- Temperature

SYSTEMIC EXAMINATION

- Cardiovascular system
- Respiratory system
- Abdomen examination
- Nervous system examination

INVESTIGATIONS

- Urine analysis
- Complete hemogram (including platelet count)
- COAGULATION PROFILE

    Prothrombin time
    Activated partial thromboplastin time
    Mixing studies(if PT/aPTT prolonged)
    Plasma fibrinogen

- Peripheral smear, reticulocyte count
- Renal function tests
- Liver function tests
- Fasting lipid profile
- Serum lactate dehydrogenase
- Viral markers- HbsAg, anti-HCV
- CD4 cell count
- Special investigations-D-Dimer, Lupus anticoagulant, bone marrow study
- ECG in all leads
- Chest X ray PA view
- USG-Abdomen & Pelvis

NO.	AGE	SEX	DURATION OF HIV(months)	DURATION OF ART INTAKE(months)	MODE OF TRANSMISSION	BLEEDING MANIFESTATIONS	CLINICAL FEATURES	SERUM CREATININE(mg/dl)	SERUM TOTAL BILIRUBIN(mg/dl)	SERUM AST(U/L)	SERUM ALKALINE PHOSPHATASE(U/L)	HAEMOGLOBIN(gms%)	PLATELET COUNT(lakhs/mm3)	PT(seconds)	APTT(seconds)	PLASMA FIBRINOGEN(180-350mg/dl)	SERUM LDH(200-400U/L)	HBsAg	AntiHCV	CD4 CELL COUNT per mm3	STAGE
1	35	F	0	0	S	nil	Ischemic stroke	0.7	0.8	46	75	8	1.96	N	N	168	198	N	N	108	IV
2	26	F	24	12	S	nil	Guillian Barre syndrome	0.9	0.9	26	110	10.2	1.7	N	N	223	243	N	N	558	IV
3	37	M	18	8	S	nil	Vacuolar myelopathy/Pulmonary TB	0.9	0.7	19	68	7.7	1.4	P	N	176	238	N	N	59	IV
4	31	F	72	24	S	nil	TB meningitis	1	1.1	24	87	6.2	1.27	P	N	218	279	N	N	43	IV
5	33	M	12	0	S	nil	Alcoholic hepatitis	0.8	8.5	238	220	11	1.65	P	P	113.4	358	N	N	699	IV
6	39	M	0	0	S	nil	Ischemic stroke/Viral hepatitis	1	6.8	118	92	10.5	0.81	P	P	78	294	N	P	88	IV
7	30	F	0	0	S	Bleed PR	Pulmonary TB/PGL/Haemorrhoids	0.9	0.6	42	149	10.4	1.8	N	N	225	139	N	N	118	III
8	40	M	120	30	S	nil	Viral hepatitis	0.8	19	270	264	9.9	1.2	P	P	88.5	271	P	N	224	IV
9	33	M	0	0	S	nil	Persistent fever/PGL	0.8	0.8	30	82	8.3	1.9	N	N	243	189	N	N	214	III
10	50	M	0	0	S	nil	Miliary Tuberculosis	0.8	0.9	36	87	10.4	1.6	P	N	218	174	N	N	302	IV
11	27	M	0	0	S	nil	Acute bronchitis	0.9	0.6	22	62	7.8	1.13	N	N	382	204	N	N	26	III
12	44	M	0	0	S	nil	Viral hepatitis	1.2	3.9	48	392	9.4	1.6	P	P	197.6	246	P	N	21	IV
13	36	M	24	12	S	nil	Ischemic stroke	0.7	1.1	29	76	13.8	0.3	P	P	396	207	N	N	96	IV
14	42	F	24	6	S	nil	Chronic diarrhea	0.6	0.7	32	53	12	1.26	N	N	179	212	N	N	342	III
15	43	M	24	24	S	nil	Pancreatitis	1.2	3.4	60	178	6.2	1.38	P	N	230	196	N	N	532	IV
16	33	M	3	0	S	nil	Sepsis/MODS	6.7	22	75	368	12	1.4	P	P	510.5	156	N	N	309	IV
17	30	M	6	4	S	nil	Pancreatitis	0.8	0.9	18	80	9.1	1.2	P	N	179	258	N	N	887	IV
18	36	M	24	1	S	nil	Fever/Sepsis/Acute kidney injury	2.3	1.7	44	142	6.5	1.04	P	N	198	214	N	N	98	IV
19	40	M	0	0	S	nil	Pulmonary TB-sputum+/	0.8	0.8	30	85	11.2	4.2	P	P	240	442	N	P	528	IV
20	65	M	0	0	S	nil	Ischemic stroke	1	0.9	38	91	9	1.52	N	P	143	199	N	N	89	IV
21	40	M	0	0	S	nil	Cryptococcal meningitis	1.3	0.8	57	79	8.3	0.9	P	P	323	138	N	N	47	IV
22	34	M	48	12	S	nil	RHD/LRI/ARDS	0.7	1	45	102	8	0.9	R	P	146	959	N	N	906	II
23	36	M	12	0	S	nil	Pulmonary TB	1	1.1	66	198	7.1	0.58	P	P	128.7	902	N	N	30	IV
24	39	M	36	0	S	nil	Pulmonary TB-relapse	1.1	0.7	28	78	12.6	1.71	P	P	168	234	P	N	386	IV
25	46	M	0	0	S	nil	Splenic abscess	1.4	0.9	35	186	4.2	1.27	P	N	194	288	N	N	31	IV
26	47	M	0	0	S	nil	Alcoholic hepatitis	1	16	120	122	10.6	1.03	P	P	234	171	N	N	116	IV
27	38	M	0	0	S	nil	Splenic candida	0.7	0.8	22	82	6.9	1.04	P	N	82	392	N	N	50	IV

NO.	AGE	SEX	DURATION OF HIV(months)	DURATION OF ART INTAKE(months)	MODE OF TRANSMISSION	BLEEDING MANIFESTATIONS	CLINICAL FEATURES	SERUM CREATININE(mg/dl)	SERUM TOTAL BILIRUBIN(mg/dl)	SERUM AST(U/L)	SERUM ALKALINE PHOSPHATASE(U/L)	HAEMOGLOBIN(gms%)	PLATELET COUNT(lakhs/mm3)	PT(seconds)	APTT(seconds)	PLASMA FIBRINOGEN(180-350mg/dl)	SERUM LDH(200-400U/L)	HBsAg	AntiHCV	CD4 CELL COUNT per mm3	STAGE
28	38	M	0	0	S	nil	Miliary TB	1.4	0.8	37	127	7.4	2.79	P	P	91	427	N	N	92	IV
29	53	F	4	0	S	nil	Acute bronchitis	0.9	0.8	26	61	9.3	1.45	P	N	178	223	N	N	355	III
30	42	M	48	12	S	nil	Alcoholic hepatitis	1.3	15	396	140	6.5	2.6	P	P	94	520	N	N	364	IV
31	66	M	60	60	S	nil	Chronic diarrhea	1	1.1	38	206	5.2	1.07	P	P	142	241	N	N	470	III
32	39	F	12	0	S	nil	Pulmonary TB-sputum +ve	1.1	0.8	34	114	4.1	1.29	N	N	164	324	N	N	245	III
33	39	M	24	0	S	nil	Cryptococcal meningitis	1	0.9	34	68	8	1.68	N	N	118.1	214	N	N	63	IV
34	49	F	0	0	S	nil	Splenic abscess	1.2	1	22	139	10.3	2.17	N	N	194	228	N	N	191	IV
35	55	M	24	24	S	nil	TB meningitis	0.9	0.9	36	101	8.8	0.75	N	N	198	397	N	N	180	IV
36	40	M	0	0	S	nil	Ischemic stroke	0.9	1	30	62	11	7.42	N	N	117	314	N	N	98	IV
37	25	M	5	0	S	nil	Chronic diarrhea	0.8	0.9	30	71	8.1	3.53	P	P	150	256	N	N	801	III
38	14	M	168	5	V	nil	Pancytopenia	0.9	0.8	20	85	2.2	0.89	N	N	142.7	592	N	N	353	III
39	29	M	48	48	S	nil	Peripheral neuropathy	1	0.8	39	76	8.8	1.4	N	N	116	134	N	N	106	IV
40	65	M	0	0	S	nil	Fever	1.4	1.8	46	88	9.5	1.15	P	N	142	314	N	N	81	II
41	34	M	24	4	S	nil	Pneumothorax/Pulmonary TB	0.9	0.7	37	65	8	1.4	N	N	223	156	N	N	112	III
42	35	M	6	6	S	nil	TB meningitis	0.7	0.9	28	120	7.4	2.17	P	N	248	213	N	N	106	IV
43	43	M	4	0	S	nil	DVT-upper limb	1	0.7	28	145	9.9	1.92	P	N	198	163	N	N	85	IV
44	28	M	24	0	S	nil	Sepsis/Cavernous sinus thrombosis	0.9	0.8	46	96	6.6	0.94	P	N	57.2	313	N	N	563	IV
45	37	F	12	12	S	nil	Drug reaction-SJS/Hepatitis	1	11	72	272	8.3	1.36	P	P	180	286	N	N	368	III
46	30	F	0	0	S	nil	Guillian Barre syndrome	0.6	0.8	36	41	9.9	2.85	N	N	227	179	N	N	123	IV
47	36	M	0	0	S	nil	Chronic diarrhea	1.5	1	30	84	10.7	2.66	P	P	162	263	N	N	365	III
48	25	F	4	3	S	nil	CMV retinitis/Pulmonary TB	1.6	0.9	45	105	7.7	1.61	N	N	128	524	N	N	68	IV
49	27	M	0	0	S	nil	Ischemic stroke	0.8	0.7	25	69	14.8	0.7	N	R	484	164	N	N	308	IV
50	42	M	0	0	S	nil	Chronic liver disease	1.9	0.9	37	101	7.3	1.91	P	N	214	294	P	N	114	IV
51	38	F	12	6	S	nil	nil	0.8	0.7	44	120	11	1.08	N	N	195	186	N	N	315	I
52	33	F	96	0	S	nil	nil	0.7	0.6	34	48	11.3	1.76	P	N	183	192	N	N	581	I
53	37	M	1	1	S	nil	Chronic diarrhea	0.9	0.7	30	45	10.6	1.92	N	N	178	159	N	N	68	III
54	32	F	0	0	S	nil	nil	0.8	0.9	36	98	10.7	1.98	N	N	190	179	N	N	475	I

NO.	AGE	SEX	DURATION OF HIV(months)	DURATION OF ART INTAKE(months)	MODE OF TRANSMISSION	BLEEDING MANIFESTATIONS	CLINICAL FEATURES	SERUM CREATININE(mg/dl)	SERUM TOTAL BILIRUBIN(mg/dl)	SERUM AST(U/L)	SERUM ALKALINE PHOSPHATASE(U/L)	HAEMOGLOBIN(gms%)	PLATELET COUNT(lakhs/mm3)	PT(seconds)	APTT(seconds)	PLASMA FIBRINOGEN(180-350mg/dl)	SERUM LDH(200-400U/L)	HBsAg	AntiHCV	CD4 CELL COUNT per mm3	STAGE
55	40	M	0	0	S	nil	RRTI/Fever	0.5	0.8	38	239	10.7	0.99	N	N	186	231	N	N	93	II
56	45	F	0	0	S	nil	Pneumonia	0.6	0.7	26	89	9	1.01	N	N	203	234	N	N	123	III
57	48	M	12	12	S	nil	nil	0.5	0.6	34	41	10	1.41	N	N	179	312	N	N	96	I
58	42	F	1	1	S	nil	nil	0.5	0.8	38	120	11	1.75	N	N	242	190	N	N	65	I
59	36	M	24	24	S	nil	nil	0.8	0.7	40	46	12.4	1.2	R	N	233	231	N	N	391	I
60	33	F	0	0	S	nil	Fever	0.6	0.7	32	46	11	2.1	R	R	256	211	N	N	466	I
61	30	M	24	24	S	nil	nil	0.6	1	58	51	9.1	1	N	N	292	379	N	N	158	I
62	31	F	72	0	S	nil	nil	0.7	0.8	24	48	10.9	1.14	N	R	238	312	N	N	579	I
63	24	F	64	64	S	nil	nil	0.5	0.7	26	56	10	1.66	P	N	192	274	N	N	285	I
64	31	M	60	60	S	nil	nil	0.8	0.6	34	56	11.7	1.28	N	N	197	231	N	N	642	I
65	29	F	60	12	S	nil	nil	0.7	0.8	28	52	11.3	1.34	N	N	180	198	N	N	709	I
66	34	F	24	24	S	nil	nil	0.7	0.7	36	54	12.3	2.4	N	R	224	196	N	N	549	I
67	43	F	3	3	S	nil	nil	0.6	0.8	44	68	11	1.88	P	N	207	193	N	N	109	I
68	36	F	36	24	S	nil	nil	0.9	0.9	28	84	10.5	1.8	N	N	221	268	N	N	381	I
69	40	M	2	2	S	nil	nil	0.8	0.7	30	64	10.8	1.3	P	N	170	223	N	N	93	I
70	30	F	0	0	S	nil	nil	0.8	0.9	31	54	11.3	2.12	N	N	312	190	N	N	479	I
71	46	M	72	72	S	nil	nil	0.6	0.7	34	46	11.4	1.03	N	N	163	234	N	N	534	I
72	35	F	12	12	S	nil	nil	0.7	0.8	31	56	10.4	2.13	N	N	178	278	N	N	125	I
73	45	M	0	0	S	nil	nil	0.6	0.9	33	36	11	2.39	P	P	190	189	N	N	423	I
74	28	F	0	0	S	nil	nil	0.8	0.6	34	39	10.8	1.24	N	N	236	256	N	N	378	I
75	40	M	24	24	S	nil	nil	0.7	0.8	36	58	11.8	1.34	N	N	196	213	N	N	403	I
76	43	M	2	2	S	nil	nil	1	0.6	46	78	12.3	1.99	N	N	185	213	N	N	67	I
77	40	M	8	0	S	nil	nil	0.8	0.9	46	52	11.5	2.6	N	N	206	186	N	N	371	I
78	34	F	34	1	S	nil	nil	0.5	1	48	63	11.6	2.4	P	N	188	172	N	N	249	I
79	35	F	24	0	S	nil	nil	0.8	0.8	31	52	10.8	1.22	R	N	204	230	N	N	441	I
80	30	F	24	0	S	nil	nil	0.6	0.8	33	46	11.9	1.1	N	N	190	224	N	N	456	I
81	47	M	82	7	S	nil	nil	0.8	0.7	32	56	11	1.86	N	N	176	178	N	N	141	I

NO.	AGE	SEX	DURATION OF HIV(months)	DURATION OF ART INTAKE(months)	MODE OF TRANSMISSION	BLEEDING MANIFESTATIONS	CLINICAL FEATURES	SERUM CREATININE(mg/dl)	SERUM TOTAL BILIRUBIN(mg/dl)	SERUM AST(U/L)	SERUM ALKALINE PHOSPHATASE(U/L)	HAEMOGLOBIN(gms%)	PLATELET COUNT(lakhs/mm3)	PT(seconds)	APTT(seconds)	PLASMA FIBRINOGEN(180-350mg/dl)	SERUM LDH(200-400U/L)	HBsAg	AntiHCV	CD4 CELL COUNT per mm3	STAGE
82	36	F	0	0	S	nil	Fever	0.8	0.9	24	144	7.1	1.9	P	P	219	190	N	N	180	III
83	33	F	0	0	S	nil	nil	0.7	0.8	32	76	10	1.32	P	N	194	212	N	N	545	I
84	46	F	2	2	S	nil	nil	1	0.8	34	52	11.1	1.23	N	N	173	187	N	N	340	I
85	32	F	82	82	S	nil	nil	0.8	0.6	42	50	13.2	2.34	P	N	222	267	N	N	791	I
86	50	M	0	0	S	nil	Chronic diarrhea	1.2	0.7	36	70	10.7	1.84	P	N	194	220	N	N	162	III
87	33	M	0	0	S	nil	Cellulitis	2.4	1.3	29	85	11.1	1.1	P	N	156	197	N	N	316	III
88	49	M	4	1	S	nil	Fever	0.8	0.7	21	80	8.2	1.57	P	P	180	268	N	N	268	III
89	47	F	48	48	S	nil	Carcinoma-cervix	1.3	0.9	25	86	9.5	1.06	P	N	91	451	N	N	370	IV
90	38	F	6	6	S	nil	nil	0.6	1	34	46	10.4	1.37	N	N	186	268	N	N	249	I
91	25	F	0	0	S	nil	Genital herpes	0.8	1.1	43	68	12.5	1	N	R	199	265	N	N	360	IV
92	33	M	0	0	S	nil	nil	0.7	1	36	42	13	1.72	N	R	265	203	N	N	423	I
93	44	F	72	0	S	nil	Recurrent oral ulcer	0.6	0.8	38	56	11.4	1.24	P	N	211	185	N	N	724	II
94	36	M	58	54	S	nil	nil	0.8	1.2	46	53	10.6	1.99	N	N	256	223	N	N	1247	I
95	47	M	2	2	S	nil	nil	0.9	1	28	45	12.4	1.87	P	N	178	167	N	N	160	I
96	34	M	36	36	S	nil	nil	0.7	1	45	92	10.6	1.45	N	N	191	171	N	N	203	I
97	37	M	27	24	S	nil	nil	0.6	0.8	43	65	11.8	1.89	N	N	260	194	N	N	158	I
98	30	F	28	3	S	nil	nil	0.8	1	24	38	11	1.6	N	N	231	174	N	N	266	I
99	34	F	82	82	S	nil	nil	0.8	0.9	42	63	10.9	1.72	P	N	188	198	N	N	709	I
100	56	M	82	46	S	nil	nil	0.7	0.8	28	41	11.6	2.1	P	N	194	170	N	N	435	I

ART-Anti-retroviral therapy,TB-Tuberculosis,PGL-Persistent generalised lymphadenopathy,CAD-Coronary artery disease,TBM-Tuberculous meningitis,

ADD-Acute diarrheal disease,TB LN-Tuberculous lymphadenitis,MODS-Multiorgan dysfunction syndrome,RHD-Rheumatic heart disease,

DVT-Deep venous thrombosis,CMV-Cytomegalovirus,SJS-Stevens-Johnson syndrome,HBV-Hepatitis B virus,PR-Per rectum,DCM-Dilated cardiomyopathy

RRTI-Recurrent respiratory tract infection,PT,APTT(N-normal,P-prolonged,R-reduced)

HbsAg, AntiHCV-(P- Positive, N- Negative)

S-Sexual, V- Vertical

No.of in patients (1 to 50)-50

No. of outpatients(51-100)-50

NO.	AGE	SEX	DURATION OF HIV(months)	DURATION OF ART INTAKE(months)	MODE OF TRANSMISSION	BLEEDING MANIFESTATIONS	SIGNIFICANT CLINICAL FEATURES AT PRESENTATION	SERUM CREATININE(mg/dl)	SERUM TOTAL BILIRUBIN(mg/dl)	SERUM ASPARTATE TRANSAMINASE(U/L)	SERUM ALKALINE PHOSPHATASE(U/L)	HAEMOGLOBIN(gms%)	PLATELET COUNT(lakhs/mm3)	PROTHROMBIN TIME(seconds)	ACTIVATED PARTIAL THROMBOPLASTIN TIME(seconds)	PLASMA FIBRINOGEN(N0rmal range 180-350mg/dl)	SERUM LDH(Normal range 200-400U/L)	HEPATITIS B SURFACE ANTIGEN(HBsAg)	ANTI-HEPATITIS C VIRUS(AntiHCV)	CD4 CELL COUNT per mm3	STAGE	D-DIMER	LUPUS ANTICOAGULANT
1	35	F	0	0	sexual	nil	Ischemic stroke	0.7	0.8	46	75	8	2	N	N	168	198	negative	negative	108	IV	Normal	Negative
2	39	M	0	0	sexual	nil	Ischemic stroke/Viral hepatitis	1	6.8	118	92	11	0.8	P	P	78	294	negative	POSITIVE	88	IV	Elevated	Negative
3	36	M	24	12	sexual	nil	Ischemic stroke	0.7	1.1	29	76	14	0.3	P	P	396	207	negative	negative	96	IV	Normal	Positive
4	65	M	0	0	sexual	nil	Ischemic stroke	1	0.9	38	91	9	1.5	N	P	143	199	negative	negative	89	IV		
5	40	M	0	0	sexual	nil	Ischemic stroke	0.9	1	30	62	11	7.4	N	N	117	314	negative	negative	98	IV	Normal	Negative
6	43	M	4	0	sexual	nil	DVT-upper limb	1	0.7	28	145	9.9	1.9	P	N	198	163	negative	negative	85	IV	Elevated	Negative
7	28	M	24	0	sexual	nil	Sepsis/Cavernous sinus thrombosis	0.9	0.8	46	96	6.6	0.9	P	N	57.2	313	negative	negative	563	IV	Elevated	Negative
8	27	M	0	0	sexual	nil	Ischemic stroke	0.8	0.7	25	69	15	0.7	N	N	484	164	negative	negative	308	IV	Normal	Negative

PATIENT CONSENT FORM

Study detail:

"HEMOSTATIC STATUS IN HIV INFECTED INDIVIDUALS"

Study centre : Rajiv Gandhi Government general hospital, Chennai.

Patients Name :

Patients Age :

Identification Number :

Patient may check (✓) these boxes .

I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask question and all my questions and doubts have been answered to my complete satisfaction. ☐

I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving reason, without my legal rights being affected. ☐

I understand that sponsor of the clinical study, others working on the sponsor's behalf, the ethical committee and the regulatory authorities will not need my permission to look at my health records, both in respect of current study and any further research that may be conducted in relation to it, even if I withdraw from the study I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from this study. ☐

I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperate with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or well being or any unexpected or unusual symptoms. ☐

I hereby consent to participate in this study. ☐

I hereby give permission to undergo complete clinical examination and diagnostic tests including hematological, biochemical, radiological tests. ☐

Signature/thumb impression:

Patients Name and Address: Place Date

Signature of investigator :

Study investigator's Name : Place Date

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI -3

Telephone No: 04425305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. Youmash V.P
PG in MD General Medicine
Madras Medical College, Chennai -3

Dear Dr. Youmash V.P

The Institutional Ethics Committee of Madras Medical College reviewed and discussed your application for approval of the proposal entitled "Hemostatic status in HIV infected individulas"No. 44062011.

The following members of Ethics Committee were present in the meeting held on 24.06.2011 conducted at Madras Medical College, Chennai -3.

1.	Prof. S.K. Rajan, MD	-- Chairperson
2.	Prof. V. Kanagasabai MD Dean, Madras Medical College, Chennai-3,	-- Deputy chairman
3.	Prof. A. Sundaram, MD Vice Principal , Madras Medical College, Chennai -3	-- Member Secretary
4.	Prof R. Sathianathan MD	-- Member
5.	Prof R. Nandhini, MD Director, Institute of Pharmacology, MMC, Ch-3	-- Member
6.	Prof. Geetha Subramanian MD DM Prof & Head , Dept. of Cardiology, MMC, Ch-3	-- Member
7.	Prof. Pregna B. Dolia MD Director , Institute of Biochemistry, MMC, Ch-3	-- Member
8.	Prof. C. Rajendiran .MD Director , Institute of Internal Medicine, MMC, Ch-3	-- Member
9.	Thiru. A. Ulaganathan Administrative Officer, MMC, Chennai -3	-- Layperson
10.	Thiru. S. Govindasamy . BA.BL	-- Lawyer
11.	Tmt. Arnold Soulina	-- Social Scientist

We approve the proposal to be conducted in its presented form

Sd /. Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information / informed consent and asks to be provided a copy of the final report

Member Secretary, Ethics Committee